### Lewy Body Pathology and α-Synuclein

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The deposition of α-synuclein as fibrillary aggregates is a hallmark lesion of a subset of neurodegenerative disorders termed synucleinopathies, including Parkinson's disease, dementia with Lewy bodies (DLB), multiple system atrophy and Hallervorden-Spatz disease. We have identified a specific phosphorylation of Ser129 of insoluble α-synuclein in synucleinopathy brains by mass spectrometric analysis and using an anti-PSer129 antibody. Immunohistochemistry by anti-PSer129 revealed abundant LBs and dot-like or curly Lewy neurites in neocortices of DLB brains. Quantitative western blot analysis showed that ~90% of insoluble α-synuclein in DLB brains is phosphorylated at Ser129, whereas ~4% of normal α-synuclein in normal rat brains is phosphorylated, which is rapidly dephosphorylated postmortem. In addition to the ~15 kDa band representing phosphorylated α-synuclein, ~22, ~29 or ~36 kDa bands that were positive both for α-synuclein and ubiquitin were detected in insoluble fractions of synucleinopathy brains, that presumably represent mono- or oligoubiquitinated α-synuclein. Some of the α-synuclein expressed in neurons or neurites of transgenic C. elegans also was phosphorylated at Ser129. Overexpression of familial PD-linked mutant a-synuclein in touch neurons or dopamine neurons elicited behavioral disturbances in transgenic C. elegans. These data suggest that accumulation of α-synuclein in a population of neurons lead to neuronal dysfunction, and posttranslational modifications, including phosphorylation and ubiquitination, play key roles in the formation of fibrous aggregates in synucleinopathy lesions.

### Nerve Injury and Pain

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While the pathogenesis of neuropathic pain is complex, it is known to depend on the degree of Wallerian degeneration which begins at the site of nerve injury with an inflammatory response. The response is inflammatory in nature because the principal cellular mediators of nerve degeneration are immune cells and macrophages recruited to the injury site in response to immunological signals mediated by proinflammatory cytokines. Cytokines represent signals generated from hours to weeks after nerve injury and have both a local painful effect at the site of nerve injury and, via retrograde axonal transport, a central effect in DRG and spinal cord on gene expression. The central effect targets transcription factors such as c-Jun and NF-kB that control the synthesis of proteins necessary for processing of nociceptive information and nerve regeneration. Tumor necrosis factor alpha (TNF) is an important cytokine in this regard in that it orchestrates the proinflammatory response to nerve injury, and, by itself, causes nerve injury and pain. TNF production is upregulated by Schwann cells and other resident endoneurial cells immediately after nerve injury and causes ectopic electrophysiologic activity in nociceptive fibers. TNF is transformed from its membrane-bound form to an active 17 kD soluble form by the activity of matrix matelloproteinases, which are integral to the processes of nerve degeneration and regeneration. Since TNF controls the degree of Wallerian degeneration and other neuropathologic events associated with neuropathic pain states, we suggest that therapies targeted inhibition of TNF or MMP activity are promising new targets for research.

### The Potential of Paraffin-embedded Archival Tissue in the Post Genomic Era

Nicoll JAR

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Neuropathology archives throughout the world contain central nervous system tissue from a very large number of individuals. This archive represents decades of collection and classification of post mortem material and biopsies. In each case there has been a neuropathological diagnosis of the disease process and there is associated clinical information. It contains very large numbers of cases of common disorders and significant numbers of rare disorders. In most cases the tissue was initially retained for diagnostic purposes and the remaining formalin-fixed paraffin-embedded tissue was archived. Although many individual and collaborative multicentre studies have benefited from this material there has been no formal, systematic organisation or use of this potentially hugely valuable resource. In particular, as DNA is analyzable in the majority of paraffin blocks, the main value is to postgenomic research to aid in the search for disease susceptibility mutations and polymorphisms and for defining correlations between genotype and phenotype. There is intense interest in collections of biological material and associated information resulting from the need to understand and utilize the rapidly increasing information emerging from the Human Genome Project. Against this background, illustrated with appropriate examples, this presentation will explore the different technologies with which information can be extracted from formalin-fixed paraffin-embedded tissue and the limitations.

### Microarrays in Brain Tumor Research

Reifenberger G

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The classification of tumors of the nervous system is based on the histological assessment of the tumor tissue according to the WHO classification system. However, the clinical course may be quite variable from patient to patient, even among patients with histologically identical brain tumors. Therefore, it is important to identify molecular markers that may be helpful for a better prediction of response to therapy and survival time. The advent of microarray-based technologies for the detection of global mRNA expression profiles and DNA copy number changes allows for the simultaneous analysis of multiple genes in tumor biopsies. We employed microarray-based expression profiling to identify novel genes that are differentially expressed between gliomas of different types or WHO grade. In addition, we devised a genomic microarray with approximately 500 bacterial arteficial chromosome clones representing all known brain tumor-associated proto-oncogenes, tumor suppressor genes, and chromosomal candidate regions. This "brain tumor chip" can be used for matrix-based comparative genomic hybridisation analysis (matrix-CGH) to determine genetic profiles of gliomas and other brain tumors. Our data clearly indicate that distinct genetic aberrations and expression profiles are associated with the

different types and WHO grades of gliomas. Furthermore, there is growing evidence that genetic differences within a given glioma group, e.g. the oligodendroglial tumors, may be associated with distinct clinical behaviour and prognosis. Thus, it apears likely that molecular analyses will supplement the routine histological assessment of brain tumors in the not too distant future.

### Vanishing White Matter

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Vanishing White Matter (VWM), also called Childhood Ataxia with CNS Hypomyelination (CACH), was defined in on the basis of characteristic MRI findings. MRI shows extensive cerebral white matter changes with progressive vanishing over time. Clinically, the disease usually has an early-childhood onset and is characterized by slow deterioration with cerebellar ataxia, variable spasticity and relatively preserved cognition. There are additional episodes of rapid and major deterioration following infections with fever and minor head trauma. These episodes may end in coma. Multiple autopsies revealed white matter rarefaction and cystic degeneration with commensurate loss of axons and myelin and preservation of oligodendrocytes. Family data were indicative of autosomal recessive inheritance. We localized a gene for VWM on chromosome 3q27 by genome-wide linkage analysis. With help of a founder effect in the east part of The Netherlands we narrowed down the critical region, of which we constructed a detailed map. Analysis of candidate genes within the critical region of 600 Kb, containing 25 genes, showed that mutations in EIF2B5, encoding the epsilon-subunit of the translation initiation factor eIF2B, cause VWM. Two distantly related patients from the south of The Netherlands did not display linkage to 3q27 but to 14q24, the region containing EIF2B2, which encodes the beta-subunit of eIF2B. In these patients mutations were found in EIF2B2. eIF2B consists of 5 different subunits, encoded by 5 different genes. In the remaining patients, we found mutations in EIF2B1, EIF2B3 and EIF2B4, encoding the alpha-, gamma- and delta-subunits of eIF2B, showing that mutations in all 5 subunit genes can independently cause VWM. eIF2B plays an essential role in initiation of translation of RNA into protein and its regulation under different conditions including hyperthermia. This probably explains the rapid deterioration of VWM patients under fever. Mutations of translation initiation factors have not been implicated in disease before. Since the detection of the genes, we have started to analyze less typical patients and have found mutations in patients with a different phenotype.

### Cell Death and Neuroprotection Following Traumatic Brain Injury

McIntosh T

The mechanisms underlying secondary or delayed cell death following traumatic brain injury are poorly understood. Recent evidence from experimental models suggests that widespread neuronal loss is progressive and continues in selectively vulnerable brain regions for months to years after the initial insult. The mechanisms underlying delayed cell death are believed to result, in part, from the release or activation of endogenous "autodestructive" pathways induced by the traumatic injury. The development of sophisticated neurochemical, histopathological and molecular techniques to study animal models of TBI have enabled researchers to begin to explore the cellular and genomic pathways that mediate cell damage and death. This new knowledge has stimulated the development of novel therapeutic agents designed to modify gene expression, synthesis, release, receptor or functional activity of these pathological factors with subsequent attenuation of cellular damage and improvement in behavioral function. This talk represents a compendium of recent studies suggesting that modification of post-traumatic neurochemical and cellular events with targeted pharmacotherapy can promote functional recovery following traumatic injury to the central nervous system.

## WORKSHOPS

### International Brain Banking Network and Web-based Data Exchange Between Brain Banks

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As neuroscience research has expanded over the past 2 decades, the demand for human brain tissue for use in biomedical research has also increased. However, the supply of quality specimens has decreased during this same period as the result of an alarming decline in the rate of autopsies. At present, autopsies are performed routinely in only a few countries, while in others, they are performed only very rarely. The scope of brain banking itself also has changed in recent years. New techniques in molecular biology, genomics, and proteomics now require tissue specimens to be collected under special conditions and at sequential time points.

The International Brain Banking Network (*http://www.intbbn.org*) was established to integrate existing brain banking networks and to provide a communication platform for data and specimen exchange. In addition to improving utilization of existing specimens, such data exchange will also improve the availability of very rare specimens that may be consolidated from banks from various countries and specimens from the emerging global "hot spots" of development of new diseases.

Structured summaries of clinical, autopsy, and neuropathological data on standardized protocols for data entry must be developed and implemented for the International Brain Banking Network. Also, standardized descriptions of the specimens must be developed to make possible exchange of specimens and consolidation of larger specimen sets. Such protocols must be precise enough to be meaningful yet remain flexible and practicable to be implemented by the international research community. Concerns of data security, quality control, and confidentiality must also be addressed.

### Brain Banking: Quality Control and Quality Assurance

Kretzschmar HA

The objective of brain banking is to deliver to researchers in various fields of neuroscience human brain tissue samples of optimal quality. BrainNet Europe (BNE) has been established as a consortium of 10 brain banks with he objective to guarantee high quality brain banking in Europe. Standardized procedures are being established in the following fields:

*1*. Evaluation of the quality of tissue processing for diagnosis. Unstained sections are shipped to the units of the network. The sections are processed using agreed routine or immunohistochemical stains. The stained sections, with a description of the procedure, are collected and the staining results are evaluated and scored by a working group. The results of the evaluation are summarized, including comments on specificity and sensitivity, registered, and reported to the members.

*2*. Evaluation of diagnostic comparability. Selected BNE cases are processed according to standard procedure and stained sections are circulated to each network member. Each member evaluates the cases and reports the results of the evaluation. The evaluations are collected, compared, summarized and communicated to the members.

*3*. Evaluation of the quality of stored tissues. The influence of storage time on tissue both in paraffin blocks as well as in freezers is poorly studied to date. In particular, the effect of "ageing" on frozen tissue to be used in research must investigated in detail.

### Modern Models of Brain Banking: What Makes Brain Banks Go Round?

Ravid R; Kamphorst W

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Brain banks are an essential repository for post-mortem specimens obtained at autopsy.The rapidly growing number of sophisticated neurobiological techniques applied on post-mortem brain tissues increases the pressure on brain banks to supply suitable autopsy material to the scientific community. The golden standard protocol of modern models for brain banks should comprise the following 7 basic entities: *i)* a well established local donor system in which consent is obtained for the use of tissues for scientific research; *ii)* compatibility of protocols for tissue procurement, management, preparation and storage for diagnostics and scientific research; *iii)* rapid autopsies with a very short post-mortem delay and a fresh dissection; these are a prerequisite for an increasing range of technical procedures and new systems such as neuronal cultures; *iv)* a generally accepted consensus on the clinical and neuropathological criteria; *v)* quality control of the dissemintaed samples; *vi)* abide by the internationally accepted guidelines for the ethical and legal aspects; and *vii)* proper safety procedures.

The rapid progress of molecular genetics has enormously helped in the elucidation of some of the causes of several major neurological and psychiatric diseases . In the coming decenia brain banks worldwide will have to collect, preserve and type DNA and RNA from brain specimens obtained via autopsy systems.

These guidelines make brain banks go round and guarantee the suitability of collected samples which in turn has its spin-off in modern scientific research.

### Banking of Control Specimens from Various Countries and Ethnic Groups and of Specimens of Non-Neural Tissues and Fluids

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To be effective in the study of disease entities, brain banks must have available specimens representing the normal, non-diseased state. What constitutes an appropriate control specimen continues to represent a problem, especially when dealing with age-related disorders. Subtle differences between normal aging and early neurodegenerative disorders have become an important research topic. Increasingly, it has become recognized that the absence of documented neurologic impairment, based on hospital chart reviews, represents a poor measure of "normality." This has led some groups to employ standardized longitudinal evaluations of neurologic function in order to document truly intact performance and early signs of impairment, an expensive and time-consuming approach. Others have used retrospective postmortem assessments of knowledgeable informants, such as care-givers and family members. There is relatively little information available regarding differences in the

spectrum of neuropathologic findings among different ethnic groups or countries of origin. This is a potentially important area for research in identifying underlying genetic and environmental factors which may play a significant role as etiopathogenetic factors. The importance of brain banks having available non-neuronal tissues and fluid samples, especially cerebrospinal fluid (CSF), has also become increasingly recognized. The comparability of postmortem CSF to clinically obtained specimens poses a significant area of concern.

### Ethical and Moral Issues Associated with Brain Donation

Benes FM, MD, PhD

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Recent advances in our understanding of the brain have pointed to the potential for breakthroughs in the development of novel treatment strategies. This, in turn, has prompted "for profit" companies engaged in pharmaceutical and biotechnology research to direct their research to the study of postmortem brain. Several ethical questions have emerged as a result of this new trend. Who owns postmortem brain tissue? Is it ethical to distribute public domain tissue obtained with federal funding to "for profit" companies? Can postmortem tissue and/or its derivatives be treated as a commodity and sold on the open market? The answers to these questions bear on the much broader issue of how a precious resource can be distributed in way that affords the greatest benefit to the public. Supported by MH/NS31862.

## POSTERS

### 001P. Brain Donation for Research, What Do People Say?

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*Introduction*. In New South Wales Australia, permission by families for transplant donation has decreased. We do know that Australians are interested in donating organs, what has not been explored is how people feel about donating brain tissue for medical research.

*Methods*. On the day of autopsy a telephone call is made to the next of kin (NOK) and they are asked if they would consider donating the brain tissue of the deceased to medical research. All reponses were recorded.

*Research*. To date 36 NOK were contacted. Twenty-two NOK gave permission for donation (61%). The main reasons given for agreeing to donating brain tissue were; exposure to an illness (themselves or deceased), the desire to help others with the same illness. The main reason that NOK didn't agree to donation was the potential impact of the decision on other family members.

*Discussion*. This positive response to the question of brain donation for research is pleasing. Most NOK who decided to donate commented that the donation allowed an "aultruistic" outcome to the death. Those who decided not to donate did so in such a way that they did not appear upset by the question. When approached by appropriately qualified people the response is more often positive.

## PLATFORM PRESENTATIONS

### 002. The Neuropathology of Pediatric Cerebral Malaria

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*Background*. Cerebral Malaria (CM), caused by Pf, is characterized by severe neurologic dysfunction, seizures, coma and death. The pathogenesis of CM is presently poorly defined.

*Methods*. We examined the brains of 51 Malawian children (7 months-9 years) with CM (32), severe malarial anemia (SMA, 7), coma of other causes (COC, 12) and 4 age-matched controls. Sections through the cerebral hemispheres, brainstem, cerebellum, pons and spinal cord were stained with H+E, LFB and with mAbs to amyloid precursor protein (APP) and fibrinogen.

*Results*. Perivascular ring hemorrhages (RH) were present in 18/32 CM, 1/7 SMA and 2/12 COC patients and were most numerous in the subcortical white matter. Areas of demyelination were present in addition to myelin destruction around RH. Axonal injury was present in 27/32 CM, 4/7 SMA and 4/12 COC cases and was seen both in association with RH and independent of vascular changes in CM. Increased permeability to fibrinogen, evidence of BBB dysfunction, was noted around apparently intact blood vessels in addition to vessels associated with RH in 14/32 CM, 2/7 SMA and 6/12 COC patients. Leaky vessels were noted with greater frequency in the white than the gray matter. These findings suggest that axonal and myelin damage, and increased permeability of the BBB, contribute to the pathogenesis of irreversible coma in pediatric CM.

### 003. Central Nervous System Infection by Cryptococcus Neoformans. An Electron Microscopy Study in a Mice Model

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*Background*. Cryptococcus neoformans (CN) can cause fatal meningoencephalitis in patient with immune deficiency. The pathogenesis of cerebral lesions observed remains obscure.

*Experimental design*. In immunocompetent mice, we recently devised a cryptococcosis model mimicking the most common clinical situation. Electron microscopy study was performed on 3 animals with severe cerebral lesions, 15 days after inoculation with a NIH52D strain of CN.

*Results*. At light microscopy we observed large meningeal capillaries containing cryptococci. Many yeasts were also present in the leptomeningeal spaces outside the capillaries. In brain parenchyma yeasts were found within abcesses and around the blood vessels. Electron microscopy showed yeasts free in the leptomeninges and capsulated yeasts within macrophages. Behind the pia matter, they were in close contact with astrocytic vascular processes. In brain parenchyma yeasts were found in pericytes, in macrophages or free in the extracellular space. They were never observed in endothelial cells.

*Discussion and conclusion*. Our observation tend to show that NC yeasts most probably are phagocytosed by monocytes in capillaries and may cross the blood brain barrier within macrophages. Alternatively, free yeasts may cross rapidly the blood-brain barrier after adhering to endothelial cells and be phagocytosed by pericytes or colonise the extra-cellular space.

### 004. A Comparison Between PCR and Bacteriology Method in Diagnosis Bacterial Meningitis

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Shaheed Beheshti Medical Sciences, Microbiological Medical Facurity, Tehran, Iran.

*Introduction*. Meningitis is one of the lethal diseases and its prompt diagnosis is of utmost importance. Current diagnostic methods (eg, bacterial cultures, cellular cultures, biochemical methods and cell count in CSF ) are not sentivite enugh. Therefore developing a new method for quick diagnosis is essential.

*Material and methods*. We extracted DNA from cerebrospinal fluid and PCR was performed by primers which amplified the 16s rRNA sequence. Microbial culture was also performed for comparison.

*Results*. We examined 51 specimens, among which 23.5% were culture positiv . By PCR method, we reported 41 positve cases. Therefore if PCR is not used, almost half of the positive cases will be missed.

*Conclusion*. We concluded that PCR is the best method of diagnosis, with high accuracy and precision for detecting microorganisms in sterile specimens in 3 to 4 hours. Since the 16s rRNA sequence has been similar in all prokaryotes, this sequence is considered appropriate for target for PCR.

## POSTERS

### 005P.The Study of Brucella Meningitis in Children From 2002-2003 in Tehran

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*Objective*. Brucella meningitis is an endemic zoonotic disease. Although it is believed that children are uncommonly involved, a number of reports from endemic areas showed a higher percentage of children involved. Clinical manifestation of childhood brucellosis are varied ranging from minimal symptoms to extreme morbidity and occasional fatality. Asymptomatic infections are also not uncommon.

*Method*. Our study was a retrospective review was made of all patients (175 cases) admitted to Mofid hospital in Tehran with meningitis from 2002-2003. Patients included were those <12 years of age and who had clinical features suggestive of bacterial meningitis.They had serum agglutination titre (SAT) for brucella of >1:160. The samples were cultured in tryptic soy broth and Thiol broth bottles. The species of brucella was determined by standard biochemical methods .

*Results*. One hundred and seventy-five children ranging in age from 6 months to 12 years were included. Most patients (76%) presented with acute symptoms of no longer than two weeks duration. The most common presenting symptoms included fever. We

found 2 children had positive CSF culture. They had history of raw milk ingestion .

*Conclusion*. Brucellosis presents in various ways and should be included in the differential diagnosis of meningitis in endemic countries. Prevention should rely on education including on boiling raw milk .

### 006P. Microorganisms Involved in Bacterial Meningitis in Children and the Role of Mycobacterium TB

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*Objective*. The specific causes of meningitis can be divided into 2 major diagnostic groups, bacterial meningitis and the aseptic meningitis syndrome. Bacterial meningitis, which may have an acute presentation (symptoms of less than 24 hours duration) or a subacute presentation (symptoms of 1-7 days' duration ), can generally be diagnosed quickly by examining bacterial methods of the sediment of the CSF (cerebral spinal fluid).

*Methods*. A retrospective study was made of medical records of all children admitted to 3 pediatrics hospitals with bacterial meningitis. Microbiological and biochemical analysis and counting cells were done in 500 CSF samples from meningitis children from 3 hospitals in Tehran (2002-2001).

*Results*. Bacterial meningitis was identified in 41 CSF samples (8.2%) were culture positive for such as Hamophilus influenzae (12.8% of total bacterial meningitis), streptococcus pneumonia (15.4%), Neisseria meningitides (12.8%) and Mycobacterium bovis (5%), Staph epidermidis (26.5%) and other bacteria. Streptococcus pneumoniae, H. Influenza, Niesseria meningitides and staph. epidermidis were the most common pathogens in 41 patients with bacterial meningitis.

*Conclusion*. Many kinds of microorganisms cause meningitis, so we have to apply sensitive methods to determine them especially H. influenzae, Mycobacterium (Tb complex) in endemic countries.

### 007P. Cellular Immune Response and Histopathological Changes in the Central Nervous System of Experimentally Infected Horses with Trypanosoma evansi Steel, 1885 (Sarcomastigophora:Trypanosomatidae)

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An histochemical and immunohistochemical study was carried out to evaluate the cellular immune response of horses experimentally infected by *T. evansi*. For this purpose the HE histochemical stain and the avidin biotin peroxidase method was used. Astrocytes were detected by using the anti-GFAP antibodies. To determine the presence and immunoreactivity of microglia cells we used anti-major histocompatibility complex II antibodies. Cellular infiltration fenotype was characterized with the aid of anti-CD3 for T lymphocytes and anti-BLA 36 antibodies for B lymphocytes. Macrophage were marked with an antibody against myeloid/histyocites antigen (clone Mac387). Lesions in the CNS of experimentally infected horses were those of a wide spread non suppurative encephalomyelitis and meningomyelitis. The severity of lesions varied in different parts of the nervous system, reflecting an irregular distribution of inflammatory vascular changes. The distribuition of T-cells, B-cells, and class II MHC antigens was examined within the CNS of equine chronically infected with *T. evansi*. Lymphoid perivascular cuffs and meningeal infiltrations were composed of predominantly T- and B-cells. Cells defined phenotypically as macrophages were rare.The parasite, *T. evansi*, wasn't identified in these horses.

## PLATFORM PRESENTATIONS

### 008. Down's Syndrome with Cerebral Amyloid Angiopathy Related Intracerebral Hemorrhage and Alzheimer's Disease

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*Background*. Cerebral amyloid angiopathy (CAA) is an important cause of spontaneous non-traumatic intracerebral haemorrhages in the elderly, accounting for 10 to 15% of all cases. The most common form of CAA is strongly associated with Alzheimer's disease (AD). Both conditions may occur in Down's syndrome.

*Methods*. Case report, routine statings and immunhistochemistry for β-amyloid, tau, ubiquitin, investigation of amyloid topography.

*Results*. We report a 47-year-old female Down's syndrome patient, who died of intracerebral hemispherical hemorrhage. Neuropathological examination reveals a relatively small brain with signs of tonsillar herniation. The age related neuritic plaque score—according to the CERAD criteria—gives a "definite" neuropathological diagnosis of Alzheimer's disease. Immunostaining for Aβ protein shows severe amyloid deposition in many blood vessels within the cortex and overlying meninges. There is microscopical evidence of previous small hemorrhages in the form of small glial scars with haemosiderin pigmentation.

*Conclusions*. CAA related hemorrhage has been rarely reported in patients with Down's syndrome and AD. This quartet of symptoms in present case gives opportunities to investigate topographical differences on distribution of amyloid pathology.

### 009. Association of a Polymorphism of the Transforming Growth Factor-β1 Gene with Cerebral Amyloid Angiopathy in Elderly

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[4] Tokyo Institute of Psychiatry, Japan.

*Background and purpose*. Recent studies have shown that transforming growth factor β1 (TGF-β1) may induce cerebral amyloid angiopathy (CAA) in Alzheimer's disease (AD), and that a T/C polymorphism at codon 10 in exon 1 of the TGF-β1 gene may be associated with concentration of serum TGF-β1. We investigated whether the TGF-β1 polymorphism is associated with risk of CAA.

*Methods*. Association between the severity of CAA and the TGF-β1 polymorphism was investigated in 167 autopsy cases of the elderly including 73 patients with AD.

*Results*. The number of the T allele positively correlated with the severity of CAA in total ($p = 0.0011$) and non-AD cases ($p = 0.0033$), but not in AD cases. There was no significant association of the polymorphism with AD.

*Conclusions*. Our results suggest that the T allele of the polymorphism in the TGF-β1 gene is a risk factor for the genetic susceptibility to CAA independent of AD in elderly.

### 010. Amyloid Angiopathy in Centenarians Brain

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[3] Dept Internal Medicine Nagoyashi Kohseiin Geriatric Hospital, Japan.

We have conducted a clinical neuropathological investigation of the brain and spinal cord of centenarians, and herein report the findings of cerebral amyloid angiopathy.

*Materials*. The brain and spinal cords from 22 centenarians were examined. These included 2 males and 20 females aged from 100 to 116 years Results: Cerebral amyloid angiopathy(CAA) were found in 16 (73%) of 22 cases. In only one case, CAA was found in the vessels of cerebral white matter; however, for the development of leukoencephalopathy of Binswanger type, arteriolosclerosis of white matter was seemed to be more important than CAA. Severe degree of amyloid deposition was found in only 2 cases. There was no relationship between the incidence and distribution of amyloid angiopathy and senile plaque. CAA-associated vasculopathies were not so often observed.

*Conclusion*. In centenarians brain, the incidence of CAA was very high, but the degree of CAA was mild and no case of lobar hemorrhage. Another factor will be needed for the development of CAA-related hemorrhage and vasculopathies. There was no relationship between the distribution and severity of CAA, and the histopathological severity of dementia of Alzheimer type.

### 011. Small Vessel Lesions Related to Cerebral Amyloid Angiopathy (CAA) and Arteriosclerosis/ Lipohyalinosis (AS/LH) are Associated with Alzheimer's Disease (AD)

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*Background*. AD constitutes the most frequent cause of dementia in the elderly population. AD-related pathology is often accompanied by vascular changes. The predominant vascular lesions in AD are small vessel changes due to CAA and AS/LH. This study was undertaken to examine the coincidence and severity of small vessel changes in relation to the development of cognitive deficits, amyloid beta-protein (A-beta) deposition and neurofibrillary tangle generation in AD.

*Methods*. We determined the clinical dementia rating (CDR) score, the A-beta-phase, the NFT-stage, the expansion of CAA and that of AS/LH in 19 different brain regions from 52 human autopsy brains.

*Results*. The expansion of CAA and AS/LH was associated with an increase of the CDR-scores, A-beta-phases, and the NFT-stages. AD cases showed a higher number of regions with CAA and AS/LH compared to non-demented cases. In AD, CAA and AS/LH together constitute small vessel lesions within the entire brain.

Conclusions: The overall expansion of CAA and AS/LH in the human brain is associated with the development of cognitive deficits due to AD. Both vascular pathologies contribute to the widespread small vessel changes in the AD brain and appear to be an integral component of the histopathological pattern of AD and AD-related neurodegeneration.

### 012. Cerebral Amyloid Angiopathy in the Elderly: Relationship with Dementia

Zekry D; Duyckaerts C; Belmin J; Geoffre C; Moulias R; Hauw J-J

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Aβ amyloid angiopathy is observed in a few families, in patients with Alzheimer's disease and in cognitively normal old persons, in association with other lesions induced by Aβ peptide and tau pathologies. We investigated the consequences of cerebral amyloid angiopathy on vessel walls dimensions by morphometry and looked for correlations with the degree of cognitive impairment in a prospective clinicopathological study (29 institutionalized elderly patients). We measured the external and internal diameters in vessels the walls of which had moderate or severe Aβ deposits and in spared microvessels of the temporal and frontal lobes. We found no differences in the external diameters. In contrast, the internal diameters of vessels with moderate Aβ deposits were smaller than those of spared vessels. The internal diameters were higher in severely affected vessels than in spared vessels. Amyloid angiopathy thus causes wall thickening and lumen reduction in early stages, wall thinning and lumen enlargement in advanced stages of the disease. We compared the severity of amyloid deposit and changes of the vessel walls with the premortem intellectual status. Severe amyloid deposits and thinner walls were linked to dementia.

## WORKSHOPS

### Transplantation After Traumatic Brain Injury

McIntosh TK, PhD

Stimulation of regeneration in the injured adult central nervous system (CNS) will likely require cellular replacement, neurotrophic factor delivery, promotion of axonal guidance/removal of growth inhibition and modulation of the immune response. Pioneering studies in neurodegenerative disease models involving transplantation of neural tissue suggest that opportunity exists for the use of cellular transplantation as a therapeutic option for repair and replacement of dysfunctional and/or dead cells following ischemic or traumatic insults to the CNS. Traumatic brain injury (TBI) is known to induce a cascade of molecular, cellular and neurodegenerative changes, leading to selective cellular dysfunction and loss in a variety of areas associated with both motor and cognitive function. To date, neural cell replacement therapy has been based on the concept that neurologic function lost to injury or disease can be improved via the introduction of new cells that can either replace the function of lost neurons, or serve as a source of trophic factors to support surviving cells and increase plasticity, survival and functional recovery. With the discovery of injury-induced neurogenesis in the adult brain, a second mechanism for cellular replacement therapy may be via promotion or enhancement of endogenous neurogenesis. This talk will summarize experimental, laboratory-based transplantation studies performed using clinically relevant models of traumatic brain injury (TBI) and provide a rationale for cell replacement therapy in the treatment of traumatic injury to the CNS.

### Is Neurotrauma an Inflammatory Disorder?

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In the immediate aftermath of a traumatic brain injury the blood brain barrier is often compromised, allowing the infiltration and accumulation of T-cells and macrophages in the parenchyma. What is not clear, in this acute phase, is whether this response is pathological and causes delayed neuronal damage or whether the inflammatory cytokines released by these cells also have some neuroprotective or regenerative actions. This is a key point because it raises the important question of whether anti-inflammatory treatments are likely to be beneficial in the acute treatment if head injury. We have previously observed that the number of T-cells in the brain declines relatively rapidly after head injury in humans and that by 48 hours there are very few to be found in the parenchyma. For this reason it seems unlikely that they have a major role to play in the development of long-term degenerative change. In stark contrast, CD68 positive macrophages can persist in the tissue for weeks or even months and do not appear to be restricted to areas of focal damage. It therefore seems that activated resident microglial cells and/or invading macrophages can maintain a sustained inflammatory process in long term survivors of head injury and this may underlie the chronic neurodegeneration that can often be seen in this patient group. Support for this idea comes from the epidemiological and pathological studies that have established a link between head injury and the subsequent development of Alzheimer pathology, which has a strong inflammatory component.

### The Neuropathology of the Glasgow Outcome Scale

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The neuropathology of head-injured patients assessed by the Glasgow Outcome Scale (GOS) will be presented.

Eighty-five cases were identified and ethical approval obtained. Twenty patients were moderately disabled (MD), 30 were severely disabled (SD) and 35 were vegetative (VS). A $Chi^2$ test for a linear trend was used for analysis.

Most SD and VS cases had had an RTA; whereas, the MD cases had a fall. Ninety-one percent of the VS cases were known not to have talked compared with 69% and 17% of the SD and MD cases. Skull fracture and evacuated intracranial hematoma were more common in SD and MD than VS, contusions were fewer in VS than in MD and SD, and of DAI with associated thalamic damage and ventricular enlargement were most common in VS. Hypoxic damage was present in 37% of VS and SD cases and there was high ICP in 71% of VS and in 57% and 25% of SD and MD. The cases have been identified from autopsy and the findings may not reflect the structural basis of the disability in those who survive. However, it may be tentatively concluded that the various degrees of disability as assessed by the GOS were brought about by similar structural changes but of differing severity.

## POSTERS

### 013P. Experimental Spinal Cord Traumatic Injury. The Investigations of the Effect of MK-801

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*Background*. Excitotoxicity due to excess of glutamate and apoptosis are probably closely interrelated and play important role in secondary injury after spinal cord trauma. It has been shown that MK-801, an antagonist of NMDA glutamatergic receptor, may block excitotoxicity.

*Methods*. Wistar rats were subjected to weight-drop experimental spinal cord injury under general anestesia. Immediately after trauma part of animals received MK-801 intraperitonealy 3 mg/kg b.m. Animals were sacrificed 7 days after trauma. Paraffin embedded spinal cords were investigated using immunohistochemistry with antibodies against Fas receptor ("death receptor"), glutamate receptor GluR-2, transporter proteins for axcitatory amino acids EAAT1-2, and GAP43 protein (neural "growth associated protein").

*Results*. The expression of the investigated antigens was not different in animals that received MK-801 from those not treated.

In not treated animals relatively numerous, apparently apoptotic cells were visible within white matter (oligodendroglia?). In MK-801 treated animals apoptotic cells were only sporadicaly observed. Expression of GAP43 was noted in the vicinity of the site of injury. Conclusions: The results confirm observations of other investigators suggesting intimate relationship of excitotoxicity and apoptosis.

The work was supported by Jagiellonian University Medical College grant no. 501/P/151/L.

### 014P. Retrospective Study of the Incidence of Deep Vein Thrombosis in Patients with Spinal Cord Lesions and Multiuple Trauma

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Deep vein thrombosis (DVP)is a frequent complication of surgery, multiple trauma, autoimmune diseases and cancer and can also occur in patients in good health. It is not easy to evaluate the incidence of this pathology in the general population because it often remains unrecognized if not searched for adequately. Data are more readily available for patients with clinical conditions at high risk because of hospital findings.

In this study we considered a population of 100 patients affected by conditions know to be carry a risk of DVP: tetraplegic or paraplegic spinal cord lesions with multiple trauma. Color-Doppler invastigations were used to identfy DVP in these patients. Prophylactic antithrombotic treatment was advised at the time of the anatomical damage for all patients except 16, of whom 3 had spinal cord lesions and 13 had head injuries. Three patients not given therapy, 2 with tetraplegia and 1 with paraplegia, developed DVP. Of the remaining 84 patients receiving prophylactic anticoagulation with low molecular weight heparin 17 developed DVP: 16 had spinal cord lesions and the other had suffered head and multiple fractures.

*Conclusions*. Immobility of the lower limbs, often accompained by a net reduction in muscle tone, is a factor predisposing to the one-set of pathologies involving the venous circulation, particularly when appropriate prophylactic anticoagulation is not employed. Because of their residual, partial mobility, patients with head injures and multiple fractures have a lower incidence of thrombosis than do patients with spinal cord damage.

### 015P. Traumatic Dissection of the Internal Carotid Artery

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The traumatic dissection of cerebral arteries is uncommon. It occurs most frequently in the second and third decades and it can be bilateral.

*Case 1*. A 14-year-old female was hospitalized with a picture of bilateral cerebral infarcts secondary to thrombosis of the superior sagittal sinus. A left carotid angiogram was performed and after three infusions, there was no filling of the arterial tree. A right carotid angiography revealed contrast media in the left anterior cerebral artery coming from the anterior communicating artery, but there was abscence of this material in the left middle cerebral artery. She became comatose and died. A dissecting hematoma in the left carotid was identified with an infarct in its area of supply. The clinical diagnosis of the preexisting condition was corroborated.

*Case 2*. A 34-year-old male suffered a blow to the neck against a knee of a player during a soccer match. Within minutes he fell and was found hemiplegic and aphasic and died some hours later. A recent pale hemispheric infarct was seen, secundary to a dissecting and obstructing hematoma of the left internal carotid artery. There was secondary brain stem hemorrhage.

The brain arteries that can be affected by trauma are the vertebral, basilar and internal carotid. The usual consequences are rupture with subarachnoid hemorrhage or, as in these cases, dissecting hematoma and infarction. No changes of cystic medial necrosis were found.

## PLATFORM PRESENTATIONS

### 016. MIB-1 Labelled Growth Fraction Fails to Predict Glioma Grade in Unrepresentative Astrocytoma Biopsies

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*Background*. Representative sampling of glioma at biopsy is essential for correct assignation of histologic grade. If sampling is inaccurate, glioblastoma multiforme (GBM) may be devoid of mitoses and necrosis and anaplastic astrocytoma (AA) may be devoid of mitoses. We hypothesized that the MIB-1 labeling index in tissue cores designed to replicate unrepresentative astrocytoma biopsies would predict the astrocytoma histologic grade.

*Methodology*. Glioma tissue microarrays (TMAs) were prepared using cores designed to replicate inadequate intraoperative glioma sampling Cores (0.6 mm diameter) were prepared from resected low grade astrocytoma (LGA), AA and GBM and were targeted to avoid mitoses, necrosis and endothelial hyperplasia. Cores were arranged in the recipient tissue block together with location and immunocytochemical controls. TMAs were immunostained with MIB-1. Individual cores were evaluated for total cell count, total MIB-1 positive cells and results were expressed as the MIB-1 % positive cells per core.

*Results*. Mean MIB-1 % values for LGA (n=47), AA (n=38) and GBM (n=46) were 0.52 (±0.82), 5.67 (±6.79) and 7.35 (±8.01); ranges 0 to 3.07, 0 to 30.08, and 0 to 29.08 respectively.

*Conclusion*. In unrepresentative glioma biopsy material where the MIB-1 counts exceed 3.07, it is possible to exclude low grade astrocytoma; however, an MIB-1 count of zero does not exclude GBM or AA. The significant overlap in the MIB1 indices between AA and GBM renders distinction between them impossible using this technique. In future glioma biopsies where histology fails to demonstrate mitoses and necrosis, we will incorporate MIB-1 labelling to ensure correct assignation of glioma grade.

### 017. Characterization and Distribution of Molecular Alterations in Gliomatosis Cerebri

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*Background*. The evaluation of the moleculargenetic basis of gliomatosis cerebri (GC) has recently begun, but the spatial distribution of the alterations was not analyzed in detail. Furthermore, the reason for the widespread infiltration seen in GC remains unclear.

*Methods*. We investigated various affected regions from 9 cases for alterations frequently found in common gliomas. Furthermore, we performed LOH analysis of the neurofibromatosis type 1 (NF1) gene, and determined the allelic status of the FGFR4, which is associated with tumor cell motility.

*Results*. Three cases had TP53 alterations in some tumor regions. EGFR amplification was found in one case. Neither MDM2 amplifications, nor mutations in the PTEN gene were detected. Five cases showed some, but not all, tumor regions with allelic losses at the CDKN2A locus, although no homozygeous deletions were seen. No LOH on chromosome 10 was seen, but all informative cases (3) had LOH in the NF1 gene. The cell motility-favoring $Arg^{388}$ allele of FGFR4 was present in a heterozygeous state in 5 cases and homozygeous in 1 case.

*Discussion*. Our data show that (I) alterations frequently found in common gliomas do occur only occasionally in GC, (II) that the distribution of the alterations is unequal in the tumor regions, (III) that the NF 1 gene is frequently affected, and (IV) that the $Arg^{388}$ allele frequency was higher in our GC cases than in common gliomas.

### 018. Distinct Methylation Profiles of Glioma Subtypes

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Gliomas are tumors of the central nervous system with a wide spectrum of different tumor types. They range from pilocytic astrocytoma, with a generally good prognosis, to the extremely aggressive malignant glioblastoma. In addition to these 2 types of contrasting neoplasms, several other subtypes can be distinguished, each characterized by specific phenotypic, as well as genotypic features. Recently, the epigenotype, as evident from differentially methylated DNA loci, has been proposed to be useful as a further criterion to distinguish between tumor types. In this study, we screened 139 tissue samples, including 33 pilocytic astrocytomas, 46 astrocytomas of different grades, 7 oligoastrocytomas, 10 oligodendrogliomas, 10 gliobalstomas samples, and 33 control tissues, for methylation at CpG islands of 15 different gene loci. We used the semi-quantitative high-throughput method MethyLight to analyze a gene panel comprising ARF, CDKN2B, RB1, APC, CDH1, ESR1, GSTP1, TGFBR2, THBS1, TIMP3, PTGS2, CTNNB1, CALCA, MYOD1, and HIC1. Seven of these loci showed tumor-specific methylation changes. We found tissue- as well as grade-specific methylation profiles. Interestingly, pilocytic astrocytomas showed no evidence of CpG island hypermethylation, but rather were significantly hypomethylated, relative to control tissues, at MYOD1. Our results show that glioma subtypes have characteristic methylation profiles and, with the exception of pilocytic astrocytomas, show both locus-specific hyper- as well as hypomethylation.

### 019. Evaluation of KIT Activation in 71 Brain Primary Tumors

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*Introduction*. The c-kit tyrosine kinase receptor pathway has been shown to be important for tumor progression particularly in gastrointestinal stromal tumors (GIST). GISTs express c-kit and 85% contain in frame deletion or insertion mutations in the exon 11 of KIT that constitutively activate the c-kit receptor. Such mutations

are responsible for the significant antitumor response to a specific antityrosine kinase treatment (Gleevec).

*Methods and results*. Immunohistochemical expression of c-kit (CD117, Dako) has first been evaluated on 71 paraffin-embedded primary brain tumors: 5 glioblastomas, 5 oligodendrogliomas (grade B), 21 malignant glioneuronal tumors (MGNT), 19 gangliogliomas, 13 ependymomas, 5 meningiomas, 2 pilocytic astrocytomas and 1 neurocytoma. c-kit immunoreactivity has been detected exclusively in glioneuronal tumors: 68% of gangliogliomas and 57% of MGNT.

We have further searched for mutations in KIT exon 11 by length analysis of polymerase chain reaction products in 3 glioblastomas, 15 MGNT and 11 gangliogliomas. No deletion or insertion mutations could be identified in any of the tumors tested.

*Conclusion*. This study demonstrates for the first time that *i)* c-kit immunoreactivity is specifically observed in brain tumors that exhibit neuronal differentiation, but that *ii)* in these tumors, KIT positivity is not associated with deletion or insertion mutations within the exon 11 of KIT and may thus be a simple reflect of neuronal phenotype.

## POSTERS

### 020P. Correlations of PTEN-Akt Pathway in Glioblastomas

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It has been repeatedly demonstrated that in glioblastoma the expression of p27/Kip.1 decreases in comparison with astrocytomas, whereas the opposite is observed for cyclin D1. Since they are both regulated by Akt, which can be activated by PTEN mutations and EGFR amplification, the relationship among PTEN mutations and EGFR amplification and p27/Kip.1 and cyclin D1 expression has been investigated in 66 operated glioblastomas. Immunohistochemistry by relevant antibodies and PCR procedures have been carried out on surgical samples of glioblastoma. PTEN and EGFR have been evaluated as wt, mutated and amplified, whereas Akt, p27/Kip.1 and cyclin D1 have been evaluated as negative (<5% positive cells), positive (5-25% positive cells) and strongly positive (>50% positive cells). The LIs were calculated in areas with the highest number of positive cells at ×1000. It was observed that there is an inverse relationship between p27/Kip.1 LI and cyclin D1 LI, but no relationship was found between Akt, p27/Kip.1 and cyclin D1 LI and the gene status of PTEN and EGFR. The hypothesis is that p27/Kip.1 and cyclin D1 expression in glioblastomas is regulated not only by PTEN and EGFR gene status, but also by other mechanisms, not excluding those focussed on the ubiquitin-proteasome system by which they can be degraded.

### 021P. Establishment of an In Vivo Rat Glioma Model for Investigations of the $GABA_A$-Receptor Subunit Expression in the Surroundings of Gliomas

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Epilepsy is a poorly understood phenomenon in patients with brain tumors. Epileptic seizures are frequently the first symptoms of gliomas. The responsible functional modifications of neuronal activity by neoplastic cells are unknown. However, an imbalance of excitation and inhibition can be observed adjacent to the neoplasia. Changes in the $GABA_A$ receptor subunit expression might contribute to this imbalance. In the present study we characterized the neuronal network in the vicinity of an in vivo established C6 glioma using histology, immunohistochemistry, and electrophysiology (acute brain slices). Tumors were induced in adult Wistar rats by stereotactic intracortical implantation of C6 cell suspension. The C6 cells were stable transfected with green fluorescent protein (EGFP), selected by Geneticin (G418) and sorted by FACS-analysis. At day 7 and 21 after tumor cell injection, animals were sacrificed and coronal brain sections were prepared. Implantation of C6 cells led to development of intracortical solid gliomas with a 100 to 200-μm zone of dispersed invasion. Over 60% of tumor cells were GFP-positive. The $GABA_A$-receptor subunits α1, α2, α3, α5 and β2 in the tumor surroundings and the resulting electrophysiological properties of this tissue are described in detail.

This study was supported by the BMBF, 01 ZZ 0105.

### 022P. Analysis of *N*-Acyl-Ethanolamine-Metabolising Enzymes in Human Brain Tumors

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*Background*. The brain contains anandamide that can activate cannabinoid-receptors. The enzymes *N*-acyl-transferase and NAPE-PLD are involved in the formation of anandamide, which in turn is degraded by the enzyme FAAH. Anandamide analogs have been shown to inhibit growth of brain tumors in rats. In human brain tumors the content of anandamide is lower compared to normal brain. Consequently the three enzymes: *N*-acyl-transferase, NAPE-PLD and FAAH may play a role in the growth of human brain tumors.

*Methods*. The enzymatic activity is analysed in meningeomas (n = 10 patients), glioblastomas and in non-tumor brain samples from same individuals (n = 10 patients). Microsomal fractions from frozen material are prepared for enzyme analysis.

*Results*. So far only a number of samples have been analyzed, expressed in mean ± SEM, pmol/min/mg protein. There appears to be lower activity of NAPE-PLD in glioma as compared to non-tumor brain (75.1 ± 88.7 versus 187.5 ± 114.8, $p<0.01$, paired t-test [n = 8]) as well as lower FAAH activity (153.9 ± 130.9 versus 450.6 ± 134.8, $p<0.001$, paired t-test [n=8]). NAPE-PLD was also lower in meningeoma (30.4 ± 15.8 [n=7]) as compared to non-tumor brain (t-test, $p<0.01$), while FAAH activity was not different between the two.

*Conclusions*. These preliminary results may indicate decreased turnover of anandamide in human brain tumors.

### 023P. Astrocytic Tumors: Distribution of the Glycoproteins of the Extracellular Matrix and its Relation to Histological Malignancy

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*Introduction*. In the central nervous system the laminin, and the fibronectin are localized in the vessels and in the meninges. The tenascin is an extracellular matrix protein prominent in special-

ized embryonic tissue and tumors. It was also described in angiogenic vessels in human astrocytomas.

*Objective*. The aim of this work was to identify the distribution of laminin, the fibronectin, and tenascin and the relationship with the malignity of the astrocytic tumors.

*Methodology*. Surgical specimens from 68 human glioma biopsies were revised, and only 38 were used. Immunohistochemical staining for GPAP, vimentin, laminin, tenascin, and fibronectin was performed.

*Results*. The laminin was positive in the glial and endothelial basement membrane identifying the perivascular space. It was positive in hyalinized and glomerulóide vessels as well as the fibronectin and tenascin. Tenascin was also positive in the pilocytic astrocytoma.

*Conclusion*. The laminin, the fibronectin, and the tenascin was increased in the hyalinized vessels independent of the histological diagnosis. Although tenascin was positive in the majority of the malignant tumor, in this work we did not find a positive relationship among this antibody and histological malignancy.

### 025P. Application of Rapid Immunohistochemical Staining as an Intraoperative Diagnostic Technique

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*Introduction*. Using the current standard intraoperative diagnostic techniques there remain a significant number of cases where a single, favoured opinion cannot be proffered and where surgical management may be influenced by the diagnosis. Using the EnVision antibody system we have developed and evaluated a protocol for rapid intraoperative immunocytochemistry on frozen section material which may be of value as an adjunct to existing techniques.

*Materials and methods*. Tissue blocks from suitable fresh tissue samples submitted for diagnostic purposes were collected. From these blocks frozen sections were cut and processed for rapid immunocytochemistry using the EnVision antibody complex and a standard panel of commonly used antibodies (CK-MNF, Cam 5.2, EMA, GFAP, synaptophysin, LCA and HMB-45). The resultant frozen section immunoprofile was then compared to the corresponding paraffin section immunocytochemistry using the same antibody panel.

*Results*. Rapid frozen section immunocytochemistry produced reliable and comparable immunoprofiles to the equivalent standard paraffin section immunocytochemistry when applied to cases of meningioma, glioblastoma, astrocytoma, oligodendroglioma and secondary carcinoma.

*Conclusions*. This study indicates that immunocytochemistry on frozen section material obtained from a variety of intracranial neoplasms is both rapid and reliable and as such may be of value as an adjunct to existing intraoperative diagnostic techniques.

### 026P. Detailed, Rapid and Inexpensive LOH Profile for Routine Diagnosis of Tumors

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There is increasing evidence that molecular aberrations may correlate well with clinical parameters in tumor patients. Related studies rely on highly sophisticated methods, require large amounts of high-quality sample material and/or depend on profound analytical expertise. An increasing number of promising data has therefore not yet resulted in routine applications. There is still a need for inexpensive, rapid, and easy to perform protocols that can reliably detect specific molecular aberrations. We have developed an approach by which a detailed loss of heterozygosity (LOH) profile of a tumor is available 3 hours post operation. DNA is obtained in a one-step procedure from routinely collected material (EDTA-blood; "quick-cut" section). A microsatellite-based marker set is used to detect the LOH status of selected genomic regions. The set targets regions recurrently deleted in gliomas and regions that have been shown or generally suspected to harbour a tumor suppressor gene. The profile is generated by 32 multiplexed PCR-reactions analysed on a single polyacrylamide gel. The overall cost for one analysis add up to less than 10 Euro. The approach presented here should proof useful in routine diagnosis of tumors. In addition, it rapidly generates valuable research data for further elucidating the relevance of specific aberrations in tumor pathology.

This study was supported by the BMBF and TMWFK, B307-01030.

### 027P. "CNS Tumor Kit": A Simple Method to Obtain Tissue for Molecular Genetics

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The increasing role of molecular genetics in neuropathology relies on the availability of "fresh" (ie, not fixed) specimens, which can be partly fixed, both for routine histology and for ultrastructural studies, and partly snap frozen and stored for molecular genetics or other purposes. This can be usually easily achieved during working hours, but sometimes for different reasons tumors are removed at night or during week-ends or festivities. In our hospital we are always able to obtain viable specimens, thanks to a simple kit we recently devised. This is made of a small cardboard box which contains a glass container filled with our choice fixative (which is Carnoy's solution ), a vial filled with glutaraldehyde for E.M., and a third vial filled with " RNA later" (T.M.), which preserves nucleic acids.

Paramedics in the operating room place proper amounts of the neopastic tissue in these 3 containers, and keep the kit refrigerated, until it can be transferred to the pathological lab, where the pathologist proceeds to further handling of the specimen.

This simple procedure makes possible to freeze samples of every CNS tumor and to perform electron microscopy, which can be sometimes most useful for practical diagnostic purposes, particularly in case of pediatric tumors.

## WORKSHOPS

### Lysosomal Diseases in Humans and in Murine Models: Similarities and Differences

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Animal models are very useful for the investigation of the pathogenesis and therapeutic means of genetic diseases. Although the naturally occurring models are very limited, large numbers of murine models of various diseases have been generated in recent years by combined homologous DNA recombination and embryonic stem cell technology. Genetic defects are the same in human patients and these models. However, clinical course and/or pathology may be different because of basic biochemical and developmental differences between these 2 species. Also, in general there is considerable variation in the phenotypes among patients due to very heterogeneous genetic background, while in murine models, phenotypes are quite uniform because of their homogenous background. In Krabbe model mouse, twitcher, peripheral nerve degeneration is far greater than that occurring in human disease. Niemann-Pick type C disease model mouse is clinically and pathologically similar to late infantile type. In human patients several phenotypes are known. Late onset juvenile type is most common and subtle differences may be present among phenotypes. In gangliosidoses models, cellular pathology may be similar but histopathology and clinical course may be different. For example, unlike human disease, the murine model of Tay-Sachs disease shows very limited clinical symptoms and relatively localized pathology. In my presentation, I plan to discuss these differences between human and mouse in selective lysosomal diseases.

### Utility of Animal Models of Lysosomal Disease

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The gene targeting technology now provides means to generate basically any mouse mutant. Mouse models can overcome various restrictions inevitably associated with human patients in studies of pathogenetic mechanisms and therapeutic trials. We will illustrate these points with our recently generated saposin A-deficient mouse. Affected mice developed progressive hind leg paralysis with clinical onset around 2.5 months. Pathology and analytical biochemistry were qualitatively identical with but generally milder than those seen in the galactosylceramidase-deficient twitcher mouse. Life span was up to 5 months. It should now be recognized that, in addition to galactosylceramidase deficiency, saposin A deficiency can also cause globoid cell leukodystrophy (GLD). Human GLD patients due to genetic saposin A deficiency might be anticipated. We then observed that affected females showed greatly improved phenotype during pregnancy. The pathological hallmark of GLD largely disappeared. The immune-related gene expression was significantly down-regulated in the brain of pregnant saposin A-deficient mice. Estrogen receptors were intensely expressed in the demyelinating area. When saposin A-deficient mice were subcutaneously implanted with time-release estrogen pellets from 30 to 90 days, the pathology was vastly improved. These findings suggest that the higher level of estrogen is a major factor in the protective effect of pregnancy. While we should be cautious in extrapolating these observations in the mouse to human disease, the phenomenon is spectacularly dramatic and estrogen administration might be worth a consideration as a supplementary treatment for some chronic genetic leukodystrophies.

### Metabolic and Molecular Basis of Peroxisomal Disorders

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The peroxisomal disorders (PDs) are relative newcomers in the arena of inborn errors of metabolism and represent a growing list of disorders in which one or more peroxisomal functions are impaired. Peroxisomes are now known to catalyze a number of indispensable functions in cellular metabolism including: *i)* fatty acid beta-oxidation, *ii)* plasmalogen biosynthesis, and *iii)* fatty acid alpha-oxidation. The cerebro-hepato-renal syndrome of Zellweger, in short Zellweger syndrome (ZS), is the prototype of the group of PDs, and ZS patients display a range of abnormalities including neurological, skeletal, ocular, and hepatological abnormalities. The PDs are usually divided in 2 subgroups with the disorders of peroxisome biogenesis (PBD) in group 1 and the single peroxisomal enzyme deficiencies in group 2. The peroxisome biogenesis disorders are not only clinically heterogeneous with such diverse entities as ZS, neonatal adrenoleukodystrophy (NALD), infantile Refsum disease (IRD), and rhizomelic chondrodysplasia punctata (RCDP) but also show marked genetic heterogeneity with the involvement of at least eleven different genes.

X-Linked adrenoleukodystrophy (X-ALD) is the main representative of the group of single enzyme deficiencies, characterized by the accumulation of very-long-chain fatty acids resulting from their impaired oxidation in peroxisomes as a consequence of mutations in the gene coding for a peroxisomal half-ABC-transporter, named ALDP. Other PDs belonging to group 2, are adult Refsum disease, hyperoxaluria type 1, and a range of other PDs. In the last few years much has been learned about the biochemistry and molecular basis of the various PDs which has led to the development of adequate methods for pre- and postnatal diagnosis. Less progress has been made with respect to the pathogenesis of the various disorders and the development of treatment strategies. Mouse models are being used to bridge the gap.

### Neuropathologic Manifestations of Peroxisomal Diseases

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Peroxisomal disorders consist of those with defects in peroxisomal biogenesis (PBD) or the import of a single matrix protein. The commonest and most severe former phenotype is the fatal infantile Zellweger syndrome due to a defect in a peroxisome biogenesis or assembly (PEX) gene, usually PEX1. The major neuropathologic consequence is pararolandic pachygyria-polymicrogyria. Elevations in very long chain saturated fatty acids (VLCFA) and deficiencies of the cell adhesion molecule L1 and doublecortin have been identified in Zellweger fetuses. Zellweger "knock-out" mice (eg, PEX

2, 5, and 11 beta) raise doubts about the pathophysiologic significance of VLCFA. Classical (type I) rhizomelic chondrodysplasia punctata (RCDP) is due to a deficiency of PEX 7, which controls the import of PTS2 proteins. Micrencephaly, decreased cerebral white matter and cerebellar atrophy (in chronic RCDP) are seen. The commonest single matrix protein import deficiency is adrenoleukodystrophy due to a loss of the ALDP gene (ABCD1) localized to Xq 28. Catastrophic cerebral dysmyelination/inflammation demyelination is seen in young boys. Identical gene defects can produce an indolent myelopathy in adults with predominant axonal degeneration (adrenomyeloneuropathy); at least one set of parent neurons (dorsal root ganglia) is preserved. VLCFA have again been implicated in ALD and AMN, but the cellular role of ALDP is still unclear. ALDP knock out mice mimic AMN more than ALD and provide evidence that peroxisomal and mitochondrial interactions are more common than previously appreciated.

## PLATFORM PRESENTATIONS

### 028. RAVINE Syndrome: a New Storage Disease?

Rodriguez D; Renouil JM; Ponsot G; Gelot A

Few years ago, a new syndrome was described in Reunion Island children: RAVINE syndrom (Réunion, Anorexie,Vomissements, Incoercibles, NEurologique) that clinically presented as a progressive encephalopathy with an early digestive phase (during the first year of age: vomiting and anorexia) and a protacted progressive neurological phase (cerebellar and brainstem signs with psychomotor impairement). MRI reveals lesions of basal ganglia,and of brainstem and cerebellar white matter that evolve to a severe atrophy. Biological screenings fail to detect any metabolic disorder.

We analyse 4 brains of RAVINE syndrom, 3 from children dead during the digestive phase and 1 from a young girl dead during the neurological phase. We observed: *i)* an intraneuronal storage that accentuates and diffuses caudo-rostrally with age; and *ii)* a white matter spongiosis that lead to sytemic fibers tracts atrophy specially in caudal braintem and cerebellum. The storage material is glycolipidic and ultrastructurally displays features of ceramide. It is associated with neuronal loss and vascular proliferation.

We assume that this represents a new syndrome both clinically and from a neuropathological point of view.

### 029. Rapidly Progressive White Matter Degeneration in 2 Siblings Dying at 4 Months of Age

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Two children of consanguineous Turkish parents dying at the age of 4 months due to a neonatal white matter disease are described. Apart from their cerebral problem, both children had dysplastic kidneys. The first child, a boy, died at three months of age with microcephaly and hypomyelination on MRI. No autopsy was performed. The younger sister had since birth feeding problems, failure to thrive, hypotonia and pyramidal tract signs. Cerebral MRI at the age of 4 weeks showed abnormally increased signal of the cerebral white matter on T2-weighted images. There was progressive neurological deterioration. Cerebral MRI at 4 months showed serious white matter degeneration . Brain autopsy showed microcephaly and cerebellar hypoplasia. The white matter of the centrum semiovale was diffusely grey and soft, caveolating at some places. On microscopical examination there was diffuse degeneration of the white matter, very pronounced in the centrum semiovale, less in other brain areas such as the pyramidal tracts and the cerebellum.

The brain stem areas were the least affected. The myelin had almost completely disappeared with some sparing of the axons. Foamy, swollen oligodendroglia were found. There was no metachromatic material present. Pallidum and putamen were not affected. DNA-analysis could exclude connatal Pelizaeus-Merzbacher disease; other white matter diseases are currently investigated.

### 030. Perinatal Aspects of Ceroid-Lipofuscinosis

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Clinical presentation of neuronal ceroid-lipofuscinosis ( CLN ) benefits from genetic approach since last years; The classical classification into infantile, late-infantile, juvenile and adult forms progressively steps and is replaced by the pattern of clinical expression of each gene defect. However, the neuropathological analysis remains in some cases the unique way to the diagnosis, specially when the clinical presentation is atypical.

We report the case of 2 families where the diagnosis of perinatal CLN was done. In one family, 2 first cousins were similarly affected by a severe neonatal encephalopathy with progressive microcephaly and EEG vanishing. In the second family, a girl, the first child of non-consanguineous parents, suffered from birth from a severe epileptic encephalopathy with progressive microcephaly; MRI investigations concluded to a lissencephaly. During the following pregnancy, cerebral MRI analysis at 33 GW revealed that the male fetus also had pachygyria, that lead to pregnancy termination. In the 4 cases, neuropathological study demonstrated extremely atrophic brains, without any giral pattern defect. This was underlayed by a severe neuronal loss in both cerebral and cerebellar cortex, whereas the remaining neurones displayed obvious intra-cytoplasmic storage. The latter has the histological features of CLN (lipidic, autofluorescent, GRODs); the genetic analysis eliminated CLN1 and 2 and further investigations are needed to identify this storage material.

### 031. An Autopsy Case of Adult GM2 Gangliosidosis, Clinically Presenting Cerebellar Ataxia and Dementia

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The patient, a 66-year-old woman began to exhibit ataxia and dysarthria at 33 years of age. Difficulty in walking developed in her 50s. At 63 years of age, dementia become evident. The finger-to-nose test was ataxic bilaterally and worse on the left side. Muscle weakness of lower limbs was observed. Deep tendon reflexes were diminished in the upper limbs and absent in the lower limbs. No pathological reflex was observed. Brain MRI showed diffuse cerebral cortical atrophy. Enzyme activity of beta-hexosaminidase (Hex) A was one fourth of normal. She died suddenly at 66 years of age. The brain weighed 1000 g, with mild atrophy of the brainstem and cerebellum. Histologically, mild to moderate loss of Purkinje cells and degeneration of the corticospinal tract was observed. Accumulation of lipid granules in the neuronal perikarya was not evident in the central nervous system. Electron microscopic observation could not revealed membranous cytoplasmic bodies in the cerebellar cortex. Genetic analysis of Hex A gene disclosed compound heterozygote, ie, Japanese common mutation

(IVS 5, -1G→T) and R499H. These mutations are known as causing infantile or juvenile form, it was interesting that this case presented with adult form clinically, and demonstrated atypical features pathologically.

### 032. The First Autopsy Case of Hereditary Adult-Onset Alexander Disease (HAAD) with a GFAP Gene Mutation (R276L)

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Unlike for infantile or juvenile Alexander Disease, a thorough autopsy of a HAAD case with a proven GFAP gene mutation has not been reported so far.

*Case*. A Japanese man who developed slowly progressive tetraparesis at 33 years of age died at 53 years of age in an ultimate bedridden state and with pseudobulbar palsy without palatal myoclonus. MRI showed prominent atrophy of the medulla oblongata (MO) and cervicothoracic cord. His younger brother with the same mutation has similar neurological features and MRI findings.

*Neuropathology*. The fixed brain, weighing 1230 g, had a remarkably small sized MO and cervicothoracic cord with a well preserved volume of the pons. Cross sections of the MO revealed unusually small and discolored pyramids, which histologically showed marked degeneration, the left one being cavitated, but with only scanty Rosenthal fibers (RFs) or astrocytosis. RFs were most frequent in the stria ter-minalis and alveus of the Ammon's horn, neither of which showed obvious degeneration.

*Discussion*. The discrepancy between the amount of RFs and the degree of degeneration of the given structures in this HAAD case may indicate that RF accumulation per se is nontoxic to neurons.

### 033. Cell Pathology of Neurons in Sporadic Animal Lysosomal Storage Diseases

Simonati A; Salvadori C; Cantile C; Rizzuto N

The central nervous system is the target of most lysosomal disorders. Intralysosomal accumulation of storage occurs primarily in neurons and is associated with pathological features, such as cell body distortion, growth of ectopic dendrites, formation of proximal meganeurites, enlargement of distal axons, and eventually cell death. Notwithstanding the recent advances in both genetics and biochemistry of lysosomal diseases the link between specific enzymatic defect and cell death is still missing. The post-mortem brain of 3 dogs and 2 cats affected with spontaneous ceroid-lipofuscinosis (2), gangliosidosis (1), and probable Niemann-Pick C (NPC; 2) was examined by morphological tools (electron microscopy and immunoistochemistry) in order to investigate possible mechanisms leading to neuronal degeneration and death. The apoptotic cell death pathway was examined by the TUNEL method, but no evidence of apoptosis was provided in any animal. Ubiquitin activation in the affected neurons was scarce in all cases but the NPC brain, whereas intense signal was observed in the dystrophic axons regardless the disease. Both lysosomal pattern and cytoplasmic stain were the features of ubiquitin immunoreactivity in NPC neurons. Indeed, NPC is not just a primary lysosomal disease but a complex storage disorder characterised by cholesterol accumulation in late endosomes/lysosomes, and abnormal intracellular trafficking. Intense ubiquitin cytoplasmic labelling may be consistent with an abnormal cell membrane recycling, as suggested in NPC.

### 034. Upregulation of Lipocalin-type Prostaglandin D Synthase in Oligodendrocytes in Mouse Models of Lysosomal Storage Diseases

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Lipocalin-type prostaglandin (PG) D synthase (L-PGDS), is a bifunctional protein responsible for biosynthesis of PGD2 and functions as a lipocalin as well. This protein is expressed in mature murine oligodendrocytes (OLs) and was upregulated in OLs of twitcher, a mouse model for genetic demyelinating disease, Krabbe's disease (Taniike et al, 2002). The L-PGDS deficient twitcher developed extensive neuronal as well as oligodendroglial apoptosis suggesting a novel role of L-PGDS to protect neurons and OLs from apoptosis. We investigated whether L-PGDS is also upregulated in other lysosomal storage diseases presenting neurodegeneration. In mouse models of Niemann-Pick type C disease, GM1 gangliosidosis, Tay-Sachs disease and Sandhoff disease, upregulation of L-PGDS were detected by RT-PCR. Strong immunoreactivity was observed in thalamus, deep cerebellar nuclei, cerebellar granular cell layer, cerebellaer white matter, and brain stem in all model mice. By double immunostaining, we found upregulated L-PGDS in perikarya and processes of OLs but not in astrocytes or microglia. In Tay-Sachs mice, we additionally found some L-PGDS-positive cerebral cortical neurons. Thus, upregulation of L-PGDS in OLs is a common cellular reaction in neurodegenerative disorders. Further investigation on the role of this protein in neurodegeneration is underway.

## PLATFORM PRESENTATIONS

### 035. Clinicopathologic Study of 123 Cases of Prolactin-secreting Pituitary Adenomas

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*Background*. Prolactinoma is the most invasive type of pituitary adenoma, and is generally believed to be well-differentiated adenoma, and to produce only prolactin (PRL). The factors related to the various biological behaviors occurring in patients of different ages and sexes await clarification. Different immunophenotypes of adenoma may show different biological behaviors and responses to medical agents, so we examined hormone production and tried to clarify the clonality of plurihormonal prolactinoma.

*Methods*. Clinicopathological factors were studied in 123 patients with prolactinomas (40 males and 83 females). The specimens were fixed in either 10% neutral buffered formalin or 70% alcohol and used for light microscopy. Alcohol-fixed tissue was used to extract deoxyribonucleic acid from 26 samples obtained from female patients for human androgen receptor gene (HUMARA) assay.

*Results*. Sixty-one cases (50%) were pure prolactinoma and 62 cases (50%) were plurihormonal prolactinoma. Spearman's rank correlation analysis revealed a significant relationship between age and serum PRL level ($P=0.0002$), age and tumor volume ($P<0.0001$), and tumor volume and serum PRL level ($P<0.0001$). Multiple regression analysis proved a significant correlation only between tumor volume and serum PRL level. The Mann-Whitney U-test revealed that prolactinomas associated with higher PRL level, larger adenoma, and higher age were significantly more invasive to the cavernous sinus and that male patients had significantly higher PRL level and larger adenoma. The HUMARA assay disclosed that 11 (85%) of 13 plurihormonal prolactinomas were compatible with monoclonal origin.

*Conclusions*. Our results disclosed that not only various hormones other than PRL can be secreted by prolactinoma, but also most multihormone-producing prolactinomas are monoclonal in origin.

### 036. Chondrosarcoma Mimicking Sellar and Suprasellar Mass

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*Objective*. To report the experience with sellar and suprasellar chondrosarcoma mimicking usual sellar mass.

*Materials and methods*. A 36-year-old male patient came to hospital for decreasing visual acuity. Brain MRI revealed round sellar and suprasellar mass with mottled enhancement with internal cystic material. The MRI favor the diagnosis of craniopharyngioma, most likely. Skull X-ray showed sellar floor erosion and widening, which favor pituitary adenoma.

*Results*. The patient underwent trans-sphenoidal approach (TSA) in a week of admission because his visual acuity was at a risk of blindness (finger count 1 m and hand movement). In the operation, sellar mass was totally removed and some of suprasellar mass was also removed as much as possible. The pathologic diagnosis revealed chondrosarcoma. Until 6 month after the first operation, suprasellar mass was not coming down. So, pterional approach was performed to remove the suprasellar mass. Intraoperatively, the origin of mass confirmed at the dorsum sellae.

*Conclusion*: We report a rare case of chondrosarcoma arising from the dorsum sellae. Peroperatively, its MRI finding suggest the craniopharyngioma and its plain skull X-ray mimicking pituitary tumor. So, we suggest that a chondrosarcoma should be included in differential diagnosis of sellar mass.

### 037. Expression of Neuronal Intermediate Filament in Normal and Noplastic Pituitary Gland

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Among type IV neuronal intermediate filaments, alpha-internexin has not been examined in anterior pituitary gland yet, though neurofilament triplets have been reported to be absent from endocrine cells of normal human anterior pituitary cells. Accordingly, we have examined distribution of α-internexin and peripherin in human developing anterior pituitary gland and in 40 cases of adenomas. In normal anterior pituitary, alpha-internexin was detected in some of synaptophysin positive endocrine cells in addition to nerve fibers. α-Internexin positive cells appeared in the embryonic pituitary during first trimester, concomitant with endocrine differentiation revealed by immunoreactivity to corticotropin and growth hormone. However, there was no correlation between the distribution of α-internexin positive cells and S-100 positive folliculo-stellate cells. In adenomas too, majority of cases (36/40) had immunoreactivity to this intermediate filament. However, number of positive cells differed in each case though growth hormone producing tumors were relatively rich in positive cells in our series.

Nestin, an intermediate filament of neural precursor cells were not demonstrate both in normal and neoplastic pituitary.

From these findings, it is suggested that α-internexin is expressed in majority of adenomas as a phenotype of anterior pituitary endocrine cells.

### 038. Primary Intracranial Melanocytic Tumor Simulating Pituitary Macroadenoma: Case Report and Review of the Literature

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*Background*. Primary intracranial melanocytic tumors are rare lesions, sellar ones being exceptional. So far, 5 melanomas and 4 melanocytomas have been described in the sellar region. We report a case of a 25-year-old white woman with a 4-year history of amenorrhea revealing an intrasellar mass with suprasellar extension suggestive of pituitary macroadenoma. Histologic examination showed heavily pigmented pleomorphic tumor cells forming cords and gland-like structures, positive for melanin, HMB-45, S-100, vimentin and negative for cytokeratins and EMA, thus demon-

strating melanocytic tumor. Extensive work-up failed to reveal any other primary site.

*Methods*. The literature is reviewed and clinico-pathological criteria are presented to distinguish between melanocytoma and melanoma. Differential diagnosis from other sellar neoplasms containing melanin pigment is discussed.

*Results*. Considerable histologic variations of both melanocytoma and melanoma render clinical and other microscopic features essential in distinguishing one from the other. Differential diagnosis of melanin-bearing sellar tumors includes metastatic melanoma, melanotic meningioma, paraganglioma, progonoma, schwannoma and ependymoma. Immunohistochemical studies are a helpful diagnostic tool and should comprise HMB-45, S-100, vimentin, EMA, cytokeratins, pituitary hormones, GFAP, NSE, synaptophysin, chromogranin and MIB-1.

*Conclusions*. Primary sellar melanocytic tumors are extremely rare lesions and present with few differential diagnoses. Deciding whether the tumor is best classified as melanocytoma or melanoma may prove difficult.

## WORKSHOPS

### Hedgehog Signaling in CNS Development and Tumorigenesis

Rowitch D; Kenney AM; Zhao Q; Kho A; Cai Z; Pomeroy S; Kohane I

Developmental programs that coordinate neural precursor proliferation and cell cycle exit are poorly understood. Signaling by the secreted factor, Sonic hedgehog (Shh), is essential for proliferation of neuronal precursors of the cerebellum, forebrain, and midbrain. Activation of the Shh pathway is also implicated in the genesis of the cerebellar tumor, medulloblastoma. Recent work has indicated a conserved role for Hedgehog (Hh) signaling in activation of G1 cyclins. However, the canonical Hh pathway, defined largely by genetic analysis, does not resemble classical mitogenic pathways. This talk will focus on signaling events downstream of Shh-patched signaling that regulate the cell cycle in neuronal precursors and will cover emerging data that indicate overlapping mechanisms in cerebellar development and genesis of medulloblastoma.

### Medulloblastoma: Pathology and New Variant

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Several histological variants of medulloblastoma are recognized. Classic medulloblastoma is composed of densely packed cells with highly hyperchromatic nuclei surrounded by scanty cytoplasm. Desmoplastic/nodular medulloblastoma shows nodular pale islands surrounded by densely packed, highly proliferative cells with dense intercellular reticulin fiber network. LOH 9q and *PTCH* mutations are more frequent in desmoplastic type medulloblastomas A periventricular progenitor cell appears to give rise to the classic form whereas the desmoplastic/nodular medulloblastoma arises from external granular cells. Medulloblastomas with extensive nodularity is characterized by intranodular nuclear uniformity and cell streaming in a fine fibrillary background. The intranodular round cells resemble the neurocytes. These neoplasms occur predominantly in children less than 3 years of age and show a highly characteristic “grape-like “ appearance at MRI. Neoplasms of this type occasionally undergo maturation and carry a better prognosis. The large cell/anaplastic variant is composed of cells with large, round and/or pleomorphic nuclei with prominent nucleoli. Large areas of necrosis, high mitotic activity and high apoptotic rate are common findings.. The large cell/anaplastic variant shows a high incidence of c-myc and less frequently N-myc amplification. However it has been recognized that large cell/anaplastic fetures can be focal and observed in both classic and desmoplastic tumors an probably represent a clonal expansion of more aggressive cells. Recently a two-tiered grading system dividing patients with medulloblastoma into anaplastic and in non-anaplastic groups. Anaplasia both focal and diffuse is associated with worse clinical outcome. Very rare variants are the medullomyoblastoma and the melanotic medulloblastoma.

### Murine Medulloblastoma Models

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Medulloblastomas (MB) and other CNS embryonal tumors, although rare in wild-type mice, commonly arise in several types of genetically engineered animals. Murine models fall into 3 general categories: *i)* animals that develop MB and/or supratentorial primitive neuroectodermal tumors (PNET) following T antigen expression due to viral CNS infection or transgene insertion; *ii)* knock-out mice with combined inactivation of both p53 and another gene regulating the cell cycle or apoptosis such as Rb, PARP, or DNA Ligase IV; and *iii)* mice with aberrant activation of the Hedgehog pathway due to loss of the inhibitory PTCH receptor or overexpression of Sonic Hedgehog. Microscopic analysis of murine MB has generally revealed divergent differentiation, as is commonly seen in human tumors, with focal expression of both neuronal and glial markers. The architecture of all reported murine medulloblastomas has been non-nodular, but some, particularly those with p53 mutations, appear to progress in terms of cytological anaplasia. Many researchers have focused on mouse MB models affecting Hedgehog signaling, because they recapitulate the genetic changes detected in a subset of human tumors. The PTCH model has already been successfully used to test drugs that inhibit the Hedgehog pathway. However, it was recently shown that medulloblastomas arising in mice lacking p53 and PARP appear to have activated Hedgehog signaling as well, suggesting that genetic lesions affecting the cell cycle and apoptosis may ultimately impact pathways commonly involved in human tumors.

## PLATFORM PRESENTATIONS

### 039. Immunostaining with INI1 Antibody Distinguishes AT/RT and PNET/Medulloblastoma

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Atypical teratoid/rhabdoid tumors (AT/RT) may be misdiagnosed as PNET/medulloblastoma and occasionally as other tumors. Characteristic histology and immunostaining including expression of EMA and SMA are seen in many AT/RT. Cases lacking typical rhabdoid cells or with equivocal immunostaining remain a diagnostic dilemma. AT/RT are characterized by the deletion or mutation of the hSNF5/INI1 gene. Antibody to INI1 is available. However, the sensitivity and specificity of this antibody for AT/RT and other CNS tumors has not been demonstrated. A series of 31 PNETs and AT/RTs were stained with INI1 antibody to determine its utility in distinguishing PNET and AT/RT. In 17 AT/RT with inactivation of INI1 at the DNA or RNA level, no expression of INI1 was detected by immunohistochemical staining. In 8 PNETs nuclear staining for INI1 was present. Six cases had histologic findings or immunostaining that made classification difficult. None of these cases had chromosome 22 deletion or INI1 mutation. INI1 antibody showed nuclear staining in all 6 cases suggesting these tumors were PNETs. Our results indicate that immunostaining with INI1 antibody correlates with the molecular findings in AT/RT and may be useful in confirming the histologic diagnosis of AT/RT. INI1 immunostaining may have particular utility in analyzing tumors with indeterminate histologic features or atypical immunohistochemical staining profiles.

### 040. Prognostic Significance of Clinical, Histological and Immunohistochemical Features in Medulloblastomas

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*Background*. Prognostic factors in medulloblastoma comprise clinical and pathobiological features. So far only few studies analysed in detail clinical, histopathological and immunohistochemical features in the same patient cohort.

*Objectives*. Systematic investigation of prognostically relevant clinicopathological features in a consecutive series of 79 medulloblastoma patients operated on between 1969 and 2000 at the Medical University Hospital of Vienna.

*Methods*. Fifty-four of the patients were males, 25 females. Age at operation ranged from 2.5 months to 21 years. Twenty patients received radiotherapy, 5 chemotherapy, 42 combined radio and chemotherapy. Histological subtype, grade and extent of anaplasia and invasion into the meninges were evaluated. Immunohistochemically, expression of neuronal (synaptophysin, NeuN) and glial (GFAP, vimentin) markers, nuclear p53 protein and the MIB1 proliferation index were determined.

*Results*. Chemotherapy and radiotherapy significantly influence the overall survival. Other clinical features (age, sex, tumor localization) do not influence the prognosis. Anaplastic tumors have a significantly shorter overall survival. Tumors invading into the meninges show a trend towards poorer outcome, whereas expression of neuronal and glial markers, p53 protein and MIB1 proliferation index have no prognostic impact.

*Conclusions*. In line with previous studies, chemo and radiotherapy and extent of anaplasia are the prognostically most significant factors in medulloblastomas.

### 041. Deregulated TP53/MDM2/p14/ARF Apoptotic Pathway is a Negative Prognostic Factor in Medulloblastoma of Adults

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*Background*. Radiotherapy modifies significantly the postoperative survival of adult patients with cerebellar medulloblastoma. Radiotherapy activates the p53-dependent apoptotic pathway. Any molecular or genetic event that interferes with this apoptotic cascade may result in a reduced response to radiation. Consequently, status and expression of genes involved in p53-dependent apoptosis may be prognostic indicators of survival. Previous data of ours demonstrated that mdm2- and p53 immunohistochemical expression are associated with worse prognosis in adult medulloblastoma.

*Methods*. We have analysed INK4a/ARF gene by multiplex PRC for deletions in 52 medulloblastoma of adult patients, treated with standard postoperative radiotherapy. INK4a/ARF encodes p14 ARF, an indirect promoter of p53 function, through mdm2 degradation. Homozygous deletions of INK4a/ARF activate the expression of anti-apoptotic factor bcl-2.

*Results*. Deletions of p14 gene were found in 25% of studied case, all desmoplastic medulloblastomas (Fisher's exact test $p=0.01$). Immunohistochemical expression of bcl-2 was more frequent in cases with INK4a/ARF deletion ($p=0.41$). Postoperative survival was shorter in deleted cases ($p=0.16$). The survival of cases having an alteration anywhere in the p53/mdm2/p14 system was definitely shorter ($p=0.03$).

*Conclusion*. This is the first report showing the prognostic role of factors linked to tumor biology in adult medulloblastoma. The association between INK4a/ARF deletion and bcl-2 expression confirms that in medulloblastoma the efficiency of the apoptotic machinery plays an important role in the response to treatments.

### 042. Immunocytochemical Study of Bone Marrow Metastases in Medulloblastoma

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*Background*. Bone marrow involvement is an unusual complication of medulloblastoma of yuong adults: it accounts of 5 to 10% of all extraneural metastases and is a cause of failure of therapy. The detection of small bone marrow deposit is difficult if based on morphologial evaluation alone, it could be useful to perform immunocytochemical analyses.

*Material and methods*. We examine bone marrow biopsy from 20 patients, age 20 to 29 years, with cerebellar medulloblastoma treated with surgical resection and cranio-spinal radiotherapy. The specimen were stained with ematoxilin-eosin and with antisera against GFAP, synaptofisin, neurofilaments, Cd3, Cd20 and cytocheratin.

*Results and conclusion*. Four patients showed focal bone marrow involvement during follow-up 5 to 20 months after surgery. In all 4 cases there were small neoplastic deposits strongly positive with GFAP and negative with synaptofisin, neurofilament and cytocheratin antisera. Bone marrow metastasis is a worse prognostic factor in medulloblastoma. The fact that metastatic cells are GFAP positive and Synaptofisin and neurofilament negative could attest that the glial component is more resitant to therapy and is the source of metastases.

### 043. c-myc Gene Amplification and mRNA Overexpression is a Genetic Marker for Poor Prognosis in Medulloblastomas

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*Object*. c-myc gene amplification and mRNA overexpression is a genetic marker for poor prognosis in medulloblastomas. Recently, it is demonstrated that gamma-catenin (plakoglobin) locates on Wnt signal pathway and activates c-myc more efficiently than beta-catenin does, and that beta-catenin, in contrast, activates cyclin D1 rather than c-myc. We investigated expression of c-myc, cyclin D1, beta-, gamma-catenins in medulloblastomas and its clinical significance.

*Methods*. This study included 24 medulloblastomas (male:female = 18:6). Immunohistochemistry was performed on paraffin sections using monoclonal antibodies. Correlation between the expression and the survival time was examined by Kaplan-Meier analysis and log-rank test.

*Results*. Cytoplasmic/membranous expression of gamma-catenin was detected in 9 (37%) medulloblastomas. Statistically, the expression correlated well with good outcome. Average survival time with gamma-catenin expression was 104 months and that without the expression was 49 months ($p = 0.008$). Nuclear staining of cyclin D1 was detected in 6 (25%). Average survival time for cyclin D1-positive group was shorter (22 months) than that of the negative group (71 months) ($p = 0.18$). Nuclear staining of c-myc and cytoplasmic/membranous staining of beta-catenin was detected in 20 (83%) and 19 (79%), respectively, showing no correlation with prognosis ($p = 0.49$, 0.74, respectively).

*Conclusions*. In medulloblastomas, gamma-catenin expression correlates with good prognosis and cyclin D1 expression has a tendency of poor outcome.

## PLATFORM PRESENTATIONS

### 045. BSE Immunohistochemical Pattern: A Comparison Between English and Italian Cases

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*Introduction*. Prion protein (PrP) is present in every case of BSE, and it's immunohistochemical detection is the most widely used confirmatory tool for BSE surveillance. Analysis of the histopathology of BSE does not indicate diversity of vacuolar pathology as seen in sheep, where the pattern of PrP immunostaining has been shown to vary, which may reflect differences in infecting strain. This study examined the type and distribution of PrP immunostaining in cattle with BSE identified through active and passive surveillance (ie, obex only) in the UK and Italy to look for any phenotype variation in this neuroanatomical region.

*Materials and methods*. In total, 113 cases were examined. Some were collected through passive surveillance (United Kingdom), and subdivided on the basis of the overall intensity of immunostaining or vacuolation. Others were identified through active programmes (United Kingdom and Italy). All cases were immunostained with Mab R145 (VLA Weybridge), and the PrP pattern subjectively assessed.

*Results*.The PrP neuroanatomical distribution was the same for all cases. The overall intensity varied, but the relative intensity anatomically within individual cases did not. PrP staining could be described as granular, glial type, intraneuronal, linear tract and aggregated. A small proportion of cases also displayed intraglial staining.

*Conclusion*. The same pathological phenotype is seen in the United Kingdom and Italy. There is no difference between active and passive surveillance cases.

### 046. Histopathology, Biochemistry and Genetics of Natural Scrapie in Sheep in 3 French Flocks

El Hachimi K; Couquet C; Deslys JP; Allix S; Dormont D; Brugère-Picoux J; Adjou K

Scrapie is a neurodegenerative disorder which belongs to the group of transmissible spongiform encephalopathies which are characterized by the accumulation in the brain of a modified, partially proteinase-resistant prion protein, $PrP^{res}$. The studies of natural scrapie isolates in France are scattered making difficult the detection of new isolates such bovine spongiform encephalopathy (BSE) agent in small ruminants. In fact, sheep and goats were as cattle exposed by feeding to contaminated meat and bone meals. The aim of the present study is to characterise the natural scrapie isolates in ewes originating from 3 different french regions (Bretagne, Limousin, Ardennes). Our results show: *i)* a clinical heterogeneity between the breeds such as the duration of the illness and the presence or the absence of prurit and tremors; *ii)* in all animals the presence of spongiosis and astrogliosis in pontic nucleus. The cerebellum was moderately affected. However, the spinal cord was severely affected only in one breed (Bretagne); *iii)* a large spectrum of $PrP^{res}$ deposits in the brain (eg, synaptic, plaque, vascular deposit); *iv)* using westen blot and ELISA (Biorad) methods that $PrP^{res}$ is present in the brain of suspected animals—no typical BSE electrophoretic profil was found in our tested samples; and *v)* the main genetic polymorphisms associated to sheep scrapie were VRQ/VRQ and ARQ/ARQ and the ARR/ARR genotype was never found in affected sheep. In conclusion, our data suggest that the 3 flocks chosen on the basis of clinical and geographical charactristics were infected by 3 various strains with no link with BSE.

### 047. Increased Corticosteroid Levels in Feces of Scrapie Inoculated Mice

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*Objectives*. In order to monitor the development of a sustained stress reaction in experimental prion disease in mice, we repeatedly determined corticosteroid levels in faeces of scrapie inoculated mice during the clinical course of scrapie disease.

*Materials and methods*. Ten female C56Bl/6J mice were inoculated intraperitoneally with RML inoculum and were compared to 10 age and sex matched controls. Between week 26 and 36 after inoculation, feces samples of inoculated animals still alive and control animals were collected weekly on 2 subsequent days. On the first day as global sample collection over a 24-hour period, on the following day in 9 defined intervals, comprising another 24-hour period. In addition, general behavior and simple motor and sensory aspects were assessed.

*Results*. Compared to control animals global corticosteroid secretion of scrapie inoculated mice increases twice: At the onset of definite clinical symptoms (1.5-fold) and 1 to 2 weeks before the final disease stage (up to 7.3-fold). The increase is paralleled by a severe disturbance of diurnal corticosteroid variation.

*Conclusions*. Development of definite clinical symptoms in scrapie inoculated mice is paralleled by a marked increase in corticosteroid secretion and disturbance of corticosteroid homeostasis. Thus, measurement of corticosteroid parameters might represent an additional tool in the early diagnosis of prion diseases.

## WORKSHOPS

### Translating the Cellular Neuropathology of Microglia into Neuroimaging Results

Turkheimer FE; Banati RB; Moran L; Duke D; Graeber MB

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The brain responds to the challenge of disease with marked changes in the functional state of its glial cells. One of the most rapid and obvious events is the activation of microglia, the brain's resident tissue macrophages—a response that is increasingly recognized to be an important, early step in the pathophysiology of traumatic, inflammatory, neoplastic and degenerative brain disease. Part of the remarkable structural and functional plasticity of microglia is the de novo expression of the "peripheral benzodiazepine binding site" (PBBS). PBBS is linked to important functions, such as immune modulation, steroid synthesis and mitochondrial activity. We have studied PBBS by integrating several data layers including gene expression arrays and immunocytochemistry, in order to identify the functional significance of the PBBS and microglial activation more generally. The PBBS is bound by the isoquinoline PK11195, which labeled with carbon-11 can be used for positron emission tomography (PET). This opens a unique window to study glial action in the living human brain. Using 11C-(R)-PK11195 PET in inflammatory and neurodegenerative brain disease as well as receptor autoradiography, we have shown that distributed regional PBBS up-regulation correlates with clinical deficit and mirrors the histologically described activation of microglia in the penumbra of focal lesions, but importantly also in the distant, anterograde and retrograde projection areas of the lesioned neural pathway as well as in structurally normal trans-synaptic areas.

### Probing with Light: How the Confocal Microscope is Transforming our View of the Brain

Page A

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The genomes of increasing numbers of organisms are being mapped and sequenced and large numbers of proteins have been identified whose functions have yet to be defined. One of the first steps in defining such functions is the precise localisation of the proteins in cells and tissues. Confocal microscopy is a key tool in this process. These microscopes have revolutionised our view of cells and tissues and have played a leading role in interpreting their complex microanatomy. The use of immunofluorescent labels coupled with the ability of confocal microscopes to provide in-focus images of relatively thick specimens (up to 100 μm) allows us to study the relationship of different antigens within a three dimensional structure. Labelling of cells, however, is not restricted to antibodies. Fluorescently labelled lectins, tracers and green fluorescent protein (GFP) have all been used in combination with antibodies to investigate a number of topics of neuropathological interest. These include the study of perivascular fluid drainage channels of relevance to cerebral amyloid angiopathy in Alzheimer's disease, the investigation of hippocampal neurogenesis and the mechanisms controlling the signalling and infiltration of leucocytes following brain injury. The use of GFP and its analogues is particularly exciting, as it enables investigators to tag almost any protein to study dynamic molecular events within living cells. So-called 4-dimensional imaging is transforming our understanding of the growth, development and pathology of the nervous system.

### MRI: New Modalities for the Investigation and Management of Neurological Diseases

Balériaux D

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Magnetic resonance imaging (MRI) has substantially improved brain imaging providing a multiplanar, non-invasive and highly sensitive method. Continuous improvements in hardware and software allow faster image acquisition, thinner image slices, higher contrast and spatial resolution. High field MRI at 3 T and more open new horizons in demonstrating unique anatomical details. MRA evolve continuously: after "black blood," "time of flight" and "phase contrast" techniques new methods using fast bolus contrast enhanced T1 weighted technique allow better and fast imaging of brain vascularisation. Dynamic MRDSA provides image acquisition with a time resolution of 2 images per second. 3T systems allow more reliable evaluation of small intracranial vessels. MRS is nowadays accessible in clinical routine thanks to software improvements: metabolic, tumoral and ischemic pathologies do benefit from those improvements. MR diffusion techniques have been made available on most clinical units: detecting early ischemic insults, evaluating brain tumors, identifying brain abscesses accurately as well as Creutzfeldt-Jakob disease are amongst the most relevant clinical applications. MR diffusion tensor images offer unique visualization of white matter fiber tracts. MR Perfusion techniques are most valuable in evaluation of brain penumbra zones or neoangiogenesis in brain tumors. Functional imaging has opened a large field of physiologic investigation. All these new modalities provide richer information about many neurological diseases: recently, image fusion allows addition of all this specific information in order to guide therapy (Neuronavigation, gamma-knife therapy) more accurately combined with other imaging modalities such as PET scan images.

### Integrated Approaches in the Investigation of Dementias

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Modern investigative techniques offer the prospect of breakthroughs in understanding. However with the exception of a handful of success stories their application to neurodegenerative disease requires their systematic integration into wider research and diagnostic frameworks. This workshop will explore some of the issues of integrating modern techniques in the investigation of dementia. The pathological investigation of diseases causing dementia has two main aims: first to establish accurate diagnosis and relate this to the natural history of disease for the purposes of public health surveillance and national health planning, second, to gain an understanding of disease that will allow the development of appropriate therapies. Tissue archiving, diagnosis and sample preparation require linkage to high quality clinical data. Prospective ascertainment of longitudinal clinical data has a very high value and will be a key resource for identification of modifier genes in neurodegenerative

diseases. Traditional approaches to morphological assessment are now being reconsidered and systematic and novel approaches to the recording and analysis of morphological data is being promoted under the term "morphonomics" which is not simply a rebranding of traditional histopathology. How this is integrated into research will be presented. Modern molecular techniques with high throughput have the capacity to generate large amounts of data on individual cases and modern integrated approaches to research are developing computer and informatics systems that allow multi-site access and facilitate peer collaboration. New approaches to data capture, data sharing and data analysis are now required. Examples of approaches to data analysis will be presented and schemes for the creation of virtual biobanks explored. These systems need linking with clinical and morphological data such that "exploration science" can be conducted with the prospect of identification of new entities. Such systems also allow the systematic capture of unusual cases which together can be highly informative and provide insights that drive wider areas of research. Finally, these aims have to be achieved within developing ethical and research governance frameworks that address issues of confidentiality, consent, safety and quality assurance. These considerations now have to be a foremost concern in research planning. The failure of public acceptance of GM crops is an important lesson to consider against proposals for stem cell research, genetic screening and use of human tissues.

## POSTERS

### 048P. Molecular Imaging for the In Vivo Measurement of Microglial Activation in Cruetzfeldt-Jacob Disease (CJD)

Moresco RM; Messa C; Tagliavini F; Panzacchi A; Giovagnoli MR; Matarrese M; Pietra L; Bertoldo A; Rizzo G; Giaccone G; Di Fede G; Mattioli C; Corbelli C; Fabio F; Bugiani O

Besta: IBFM-CNR, University of Milan Bicocca, San Raffaele Hospital, Scientific Institute Besta, Milan, University of Padova, Italy.

The expression of peripheral type benzodiazepine receptors (PBR) significantly increases in pathological conditions with microglial activation. The selective PBR antagonist {11 C}PK11195 has been used for the in vivo PET monitoring of microglial activation. By this method we investigated the activation of microglial cells with occurs in CJD following the deposition of the prion protein. Two patients with sporadic CJD (CJD was probable in one patient while definite in the other according to a PRNP Val 210lle mutation) and one patient with the new variant form were compared with 3 age-matched controls. The in vivo binding of {11 C}PK11195 (as evaluated by the simplified reference tissue model) was significantly increased in CJD patients, particularly at the level of putamen, caudate nucleus, thalamus, occipital cortex, and cerebellum. Compared with MRI findings, increased PRB binding was observed in structures also hyperintense in T2, PD and FLAIR images. This study provides an in vivo evidence of microglial activation in CJD patients, and supports the view that MRI hyperintensities are related with the presence of activated microglia. Furthermore, it emphasizes the role of molecular imaging in the clinical evaluation of CJD.

## PLATFORM PRESENTATIONS

### 049. Reproducing Human Glioblastoma in Nude Mice

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Development of rodent intracranial models of glioblastoma (GBM), the most common of human malignant gliomas, is critical both for studying its biology as well as testing novel therapeutic strategies. Genetic engineering of mice through transgenic and knockout approaches have been widely used to breed mice that spontaneously or conditionally develop GBM. This approach holds the advantage of working with immunocompetent organisms allowing testing of therapies influenced by or dependent upon host immune response. However, the majority of the genetic modifications thus far imposed on mice to cause GBM development are uncommon in humans, questioning the relevance of the associated models. Introduction of tumorigenic human GBM cell lines into immunocompromised hosts have also been widely used. These cell lines, upon sustained culturing, however, change morphology and show reduced invasiveness once grown in vivo, challenging the relevance of this model. We were able to produce tumors faithfully recapitulating the morphology and invasiveness of human GBM, by propagating GBM surgical specimens as heterotopic (subcutaneous flank) xenografts through serial passaging in nude mice, for as long as 2 years, and injecting intracranially dissociated cells following brief, ex vivo treatment with collagenase of excised, flank-grown GBM in 3 unique cases. Characterization of these 3 GBMs indicates retention of the genetic alterations present in the patient surgical specimens, including EGFR amplification. We anticipate that this simple and faithful GBM model will see much use in neuro-oncology research.

### 050. Diagnostic Model with Molecular Investigations in Brain Tumor Stereotactic Biopsy (STB)

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Our main goal was to determine diagnostic model to obtain the fullest possible information of tumor and expected response to radiotherapy, to allow for the most efficient treatment.

The model suggested has been based on diagnoses of 150 patients subjected to STB. Procedures involved to obtain smears, fix them and to process the rest of material enrich the examinations by immunohistochemistry (IHCH) and molecular examinations. The latter are suitable for determination of real tumor malignancy and for projecting efficiency of brachytherapy based on the number of mRNA copies encoding BCL2 and BAX proteins. The expression of genes examined was determined by RT-QPCR (Taq-Man) technique using ABI PRISM 7700 sequence detector and hybridisation probes marked with FAM and TAMRA fluorescent markers.

The classical tumor grading and IHCH determination of proliferative activity does not provide for evaluation of tumor biology. When smears were fixed in alcohol and bioptates were shortly fixed in neutralised formalin, it was possible to perform molecular examinations (RNA detection and determination of transcriptive activity of genes).

The molecular examinations allow for prognosticating the course of disease based on analysis of the exceptionally fine material. It was found that BAX expression when higher than BCL2- provides good prognosis for radiotherapy.

### 051. D110 Immunoreactivity in Glioblastoma: Its Association with Expression of Hypoxia-Related Proteins and Overall Survival

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*Background*. Recently, immunoreactivity (IR) of monoclonal antibody D110 against a so far unidentified epitope has been observed in multiple sclerosis (MS) brains. D110 IR is seen in a specific subset of MS lesions mimicking hypoxic damage (Lassmann et al, *Brain* 2003, in press).

*Objectives*. To investigate D110 IR in glioblastoma and its association with expression of hypoxia-related proteins and prognosis.

*Methods*. Evaluation of D110 IR in a consecutive series of 114 glioblastomas operated at the Medical University Hospital of Vienna between 1995 and 1999. Median age at initial surgery was 60 years (range 35-78 years). D110 IR was evaluated qualitatively and semiquantitatively. Semiquantitatively assessed D110 IR data were correlated with expression of hypoxia inducible factor 1alpha (Hif 1$\alpha$), vascular endothelial growth factor (VEGF), and patient survival using univariate and multivariate statistical analysis.

*Results*. Focal and/or perinecrotic D110 IR in tumour tissue was shown in 39.6% of cases. D110 IR in infiltrating cells was shown in 83.3% of cases, apparently of microglial differentiation. We found no statistical correlation of D110 IR and expression of Hif1a and VEGF. Presence of D110 immunoreactive infiltrating cells correlated positively with overall survival in univariate analysis. In multivariate analysis, only a high postoperative Karnofsky index remained an independent factor of favorable prognosis.

*Conclusions*. In glioblastoma, focal/perincrotic D110 IR is detectable in a fraction of cases. The majority of glioblastomas shows D110 IR in infiltrating, apparently microglial cells. D110 IR does not correlate statistically with expression of hypoxia related proteins. Presence of D110 IR univariately correlates with favorable survival but is not an independent prognostic factor.

### 052. Loss of Heterozygosity of a Locus on Chromosomal Region 17p13.3 in Astrocytic Tumors—Association with Histopathological Grades, p53 Imunoreactivity and Proliferation Index

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*Introduction*. There are several tumor suppressor loci reported on the short arm of chromosome 17, of these, p53 on 17p13.1 is the most prominent. However, distal to this p53 locus, at the 17p13.3 region there are several putative tumor suppressor genes notably HIC1, OVCA1, OVCA2 and ABR. This study was undertaken to

study alterations at the 17p13.3 region in astrocytic tumors of various grades and attempt to define its role in these tumors.

*Methods*. Heterozygosity status of p53 (17p13.1) and 17p13.3 (D17S379 locus) were studied in 45 astrocytic tumors of WHO grades II, III and IV using PCR based microsatellite and restriction fragment length polymorphism (RFLP) of DNA extracted from microdissected paraffin embedded sections. Sequencing for p53 gene was done in 5 of the cases. Immunohistochemical staining for p53 protein immunoreactivity and estimation of MIB-1 labeling index was done in 40 of these 45 cases.

*Results*. Loss of heterozygosity (LOH) at 17p13.3 region (D17S379 locus was significantly associated with astrocytic tumors of higher grades namely, anaplastic astrocytoma and glioblastoma ($p=0.02$). In contrast, LOH of p53 locus showed no such association with tumor grade and was observed in both low and high grade astrocytic tumors. The LOH of 17p13.3 was found to be independent of the status of p53 gene. This was further substantiated when no abnormalities were revealed on sequencing the exons 5 to 9 of p53 gene from 5 tumors with LOH of 17p13.3 but no LOH of p53.

It was also observed that p53 immunopositivity was significantly associated with LOH of 17p13.3 (Fisher's exact two tailed $p=0.012$; odds ratio 12) but not with LOH of p53 (Fisher's exact 2-tailed $p=0.324$; odds ratio 2.24).

Further, proliferation index as determined by MIB-1 LI was found to be significantly higher in tumors with LOH of 17p13.3 than those with no LOH (Fisher's exact 2-tailed $p=0.001$). This was particularly true for high grade tumors for which LOH of this locus was demonstrated in more than 40% of cases. No such association was noted with LOH of p53. However, interestingly, p53 protein immunopositivity was associated with increased MIB-1 LI.

*Conclusions*. Thus, the present study shows the role of alterations at the 17p13.3 region (D17S379 locus) in the genesis of high grade gliomas. It also demonstrates for the first time an association of alterations at this locus (harbouring putative tumor suppressor genes) with increased cell proliferation. This therefore has important potential for being used as a molecular marker to define tumors with more aggressive phenotype within a histological group. The study also points to a new molecular correlate for p53 immunopositivity in tumors without mutations in the p53 gene. The mechanism of p53 immunopositivity and LOH at 17p13.3 observed in our study can however only be speculated upon. Further work is required to identify the exact affected genes at this locus and their role in the pathways of glioma genesis.

### 053. Comparative Neuropathological Evaluation of MR-based Experimental Continuous and Cyclic Cryoablation and LITT

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Minimally invasive brain tumor therapy includes cryosurgery with either continuous or freeze-thaw-cycle application, both with the advantage of clear intraoperative imaging by MR. To facilitate clinical application, we have examined the time course of tissue effects in comparison with laser-induced interstitial thermotherapy (LITT). Interstitial cryolesions were induced in one year old merino-sheep in the parieto-occipital region. Twenty-four animals were examined by MRI and 16 of these neuropathologically in intervals ranging from 2 hours to 18 weeks. Frontal slices of the fixed brains and microscopic sections stained with a number of histological and immunohistochemical methods were quantitatively evaluated with a Zeiss KS 400 digital system. Circumscribed cortical and subcortical lesions were visible after 2 hours already by plasma exsudates, small hemorrhages and perifocal edema but became fully apparent in the course of the first days. Resorptive changes resulted in macrophage containing cystic lesions. In comparison to LITT, the zones of marginal granulation and fibrous tissue and perifocal gliosis were smaller, often with intact neurons and ectatic blood vessels conspicuously decorated by laminin. Quantitative in vivo and in vitro data correlated in individual cases despite a great variation both in the area of necrosis (mean of 123 $mm^2$) and in the average width of the marginal zone (98-263 $\mu m$). It is concluded that cryotherapy-specific changes include pronounced and immediate blood-brain-barrier disturbances but a less extensive marginal tissue reaction. Furthermore, local tissue factors such as gyral architecture and tissue heterogeneity have to be taken into account in the operative setting.

## POSTERS

### 054P. Immunohistochemical Expression on Non-Neoplastic Microglia in Grade I-III Glioma

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*Background*. Recent technical developments have made available better methods to identify microglial cells, the resident immune cells of the central nervous system. However, the exact role of microglia in glioma has remained unknown.

*Methods and results*. We have investigated the immunohistochemical of Major Histocompatibility Complex (MHC) class II molecules (CR3/43) and of the microglia/macrophages marker CD68 in 23 paraffin-embedded astrocytic gliomas (9 pylocitic Grade I, 8 diffuse Grade II and 6 anaplastic Grade 3) to answer the question if there is a correlation with glioma grade. Microglia expressing MHC class II were common in diffuse and anaplastic gliomas, but rare in pylocitic tumors, and there was a significant difference (ANOVA – Tukey multiple comparison test) when comparing each group (CD 68: Grade I versus Grade II $p<0.001$, Grade I versus Grade III $p<0.001$, Grade II versus Grade III NS; CR3/43: Grade I versus Grade II $p<0.001$, Grade I versus Grade III $p<0.01$, Grade II versus Grade III NS).

*Conclusions*. Microglia in Grade II and III gliomas are well equipped to function as antigen presenting cells. Neoplastic cells acquire the capacity to down-regulate microglial MHC complex expression and may induce T-cell clonal anergy through aberrant expression of MHC class II molecules.

### 055P. Radiation Effect Versus Recurrent Glioblastoma: A Clinicopathological Review of 63 Patients

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*Background*. Radiation effect refers to the tissue injury that results from radiotherapy and includes several histological changes such as necrosis, vascular alterations, and gliosis with atypia. The symptoms of radiation effect and tumor recurrence are similar; moreover, the 2 entities cannot be distinguished using conventional imaging. Our study aims to determine whether histological evaluation of a specimen as radiation effect or tumor recurrence has prognostic significance.

*Methods*. We identified all glioblastoma patients who underwent a second surgery following radiotherapy at UCSF between 1989 and 2001. Cases with insufficient clinical or pathology material were excluded. Two pathologists evaluated specimens, and assigned each specimen to a final category: 1) recurrent tumor, 2) no recurrent tumor (radiation effect only), or 3) histological findings of uncertain significance. Clinical information including patient demographics, treatment, and clinical course were obtained from patient files.

*Results*. There were 63 patients with a median age of 51 years. Thirty-one patients were in category 1, 23 in category 2, and 9 were in category 3. Mean PFS was 6.4, 6.3 and 9.1 months for categories 1,2, and 3, respectively. The OS was 32.8 months for category 1, 31.9 for category 2, and 25.0 months for category 3. There was no significance among the groups in terms of PFS or OS.

*Conclusions*. Our results imply that many factors affect the outcome of patients with glioblastoma, and confound the predictive value of histopathological evaluation of post-radiotherapy specimens. A larger study with more uniform patient population is currently being planned to validate the results of this study.

### 056P. Cytokinesis-block Micronucleus Assay in Irradiated Human Glioma Cell Lines

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*Background*. Brain gliomas are mostly radioresistant tumors. Rapid proliferation of glioma cells between fractions is one possible explanation for this property. Aim of the study is to evaluate cytogenetic alterations in irradiated brain glioma cell lines and their relation to proliferative activity of tumor cells in vitro.

*Methods*. Nine human glioblastoma cell lines obtained from DSMZ (Germany) were irradiated (Co-60) over a dose range of 0-10 Gy at a dose rate 0.8 Gy/min. Cytokinesis-block micronucleus assay was performed to quantitate cytogenetic alterations. The number of micronuclei (MN) per binucleate cell was scored. Proliferative activity was measured prior and after irradiation with histone mRNA in situ hybridization.

*Results*. The number of spontaneous MN ranged from 0.17 to 0.613 (mean: $0.29 \pm 0.14$). After iradiation increase of MN count in range of 0.312 to 2.241 (mean: $0.98 \pm 0.68$) was found at 10 Gy. Histone labelling index (HLI) ranged from 12.2 to 42.5% (mean: 20.1 $\pm 9.8$). There was a slight positive correlation ($r = 0.24$) between MN count and HLI after irradiation.

*Conclusions*. Gliomas are extremely heterogenous in regard to cytogenetic effects of irradiation. Positive correlation between HLI and induction of MN after irradiation suggests the role of factors other than continuing proliferation in radioresistance of gliomas.

### 057P. FISH-ing for 1p/19q Aberrations in Human Gliomas

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*Background*. Fluorescence in Situ Hybridization (FISH) for detection of genetic aberrations is well established in research and clinical practice. The importance of 1p/19q deletions for survival and increased chemosensitivity in oligodendrogliomas is evident. Many investigations deal with PCR-based microsatellite analysis; a technique not always feasible in the diagnostic laboratory. Therefore, we have implemented a FISH procedure for 1p/19q LOH on formalin fixed and paraffin embedded tissue.

*Methods*. Forty gliomas were studied (5 oligodendrogliomas, 5 astrocytomas, 5 oligoastrocytomas, all WHO II; 5 anaplastic oligodendrogliomas, 5 anaplastic astrocytomas and 5 anaplastic oligoastrocytomas, all WHO III; and 10 glioblastomas, WHO IV). Tissues from 4 brain biopsies without tumor served as controls. Telomere probes for 1p and 19q (Vysis) were applied simultaneously. Approximately 150 cells were counted in a triple-pass filter in each tumor section.

*Results*. The study demonstrated significant more LOH 19q and 1p in gliomas with a significant proportion of oligodendroglioma components, whether pure oligodendrogliomas or mixed tumors; and independent of WHO grading.

*Conclusions*. Our results are in accordance with other's and pose more questions, such as the number of cells to be counted, number of chromosomes lost during tissue processing; and where to define the cut off level for deletions. If genetic aberrations are to be a diagnostic tool with therapeutic consequences, criteria for the interpretation of the result should be agreed upon.

### 058P. A Possible Pro-Drug in the Cerebral Glioma Systemic Treatment: Butyrric Acid Cholesterol Ester

Calderoni S[1]; Guido M[1,2]; Re S[1]; Brioschi A[1]; Zenga F[2]; Zara GP[2]; Pradotto L[1,2]; Ugazio E[2]; Gasco MR[3]; Mauro A[1,2]

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*Objectives*. Evaluation of cytotoxic, pro-apoptotic and differentiating activities of CholBut (Cholesteryl Butyrate)-SLNs in an in vitro and in vivo experimental model of rat brain glioma.

*Methods*. Rat and human glioma cell lines were treated with both Na-Butyrate and CholBut-SLN (0.05-3 mM) from 24 to 72 hours. Using stereotactic technique C6 glioma cells were implanted in rat brain and after 13 to 15 days animals were systemically treated with CholBut-SLNs. Brain slices were obtained after 2 and 4 days. Cytotoxicity, apoptosis and morfological changes were evaluated both in vitro and in vivo.

*Results*. In vitro CholBut-SLNs induce much more apoptotic cell death compared to Na-Butyrate at the same concentrations. FACS analysis and annexin V labelling clearly showed a marked increase in the number of cells with DNA fragmentation, a block in G2-M

phase. In vivo animals were not treated with Na-Butyrate because its systemic toxicity; CholBut-SLNs given sistemically did not have any adverse event and showed an increase in apoptotic cell death in tumor tissue.

*Conclusions*. CholBut-SLNs showed pro-apoptotic and anti-proliferative activities both in vitro and in vivo and could represent a potential and effective adjuvant agent in treatment of human malignant glioma.

### 059P. Active MMP-9 Expression is Associated with Primary Glioblastoma Subtype

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*Purpose*. Glioblastoma multiforme (GBM) is an aggressive cancer characterized by extensive brain invasion. Matrix metalloproteinase (MMP)-9 plays a major role in this process. GBMs can be divided into 2 subtypes based on distinct clinical and molecular features. Primary GBMs arise de novo and frequently over-express the epidermal growth factor receptor (EGFR) and its ligand-independent variant EGFRvIII; secondary GBMs progress from a lower grade glioma and commonly harbor p53 mutations. Because EGFR signaling promotes MMP-9 expression and activation in other cancer cell types, we analyzed whether MMP-9 was associated with primary GBM subtype.

*Experimental design*. Autopsies were performed on 20 GBM patients and MMP expression was assessed by gelatin zymography in the tumor and the adjacent normal brain. EGFR, EGFRvIII, p53 and activated MAPK/ERK were assessed by immunohistochemistry and associations between molecular phenotype and MMP-9 expression were analyzed.

*Results.* Latent MMP-9 was detected in 90% of tumors and active MMP-9 was found in 50% of tumors. MMP-9 was not detected in any of the normal brain samples ($p<0.001$). More importantly, primary GBMs were significantly more likely than secondary GBMs to contain active MMP-9 (69% of primary and 14% of secondary GBMs contained active MMP-9; $p = 0.027$). Active MMP-9 was observed in 73% of EGFR over-expressing/p53 wild-type staining tumors but only 20% of EGFR negative/aberrant p53 staining tumors ($p = 0.072$). Active MMP-9 expression was even more strongly correlated with EGFRvIII expression, occurring in 83% of the EGFRvIII immunopositive tumors, but none of the EGFRvIII negative tumors ($p = 0.0004$). ERK activation was also strongly correlated with EGFRvIII expression ($p<0.0001$) and with MMP-9 activation ($p = 0.003$).

*Conclusion*. These results identify a novel association between MMP-9 activation and primary GBM subtype and suggest that primary GBM patients, especially those whose tumors express EGFRvIII, may benefit from anti-MMP therapy.

### 060P. Is p53 a Predictor in Astrocytoma Progression? An Immunohistochemical Analysis of 68 Cases

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*Background*. Concept of predicting biological behavior of tumors based upon tumor markers has long been a hope of oncological community. p53 often referred to as "guardian of the genome" appears to play an integral role in multistep pathway of tumorigenesis

*Methods*. A series of 68 patients (23 WHO grade II, 13 grade III, and 32 grade IV) with astrocytomas of various grades including 41 recurrent tumors were evaluated. In each case, a minimum of 1000 cells was counted and p53 score was expressed as percentage of immunolabeled nuclei. Recurrence was established by clinical and/or radiological parameters.

*Results*. Of the 21 grade II astrocytomas that recurred, 5 (24%) were in same grade, 11 (52%) progressed to grade III and 5 (24%) became grade IV. Six of the grade III, astrocytomas that recurred, 5 (83%) progressed to glioblastomas. As expected, all 14 glioblastomas recurred to the same grade. High-grade tumors had higher p53 score as well as trend towards positivity. Mean p53 values were higher at recurrence and there was also a trend towards acquisition of p53 positivity at recurrence. Median time to recurrence was less in each grade for p53 positive tumors as compared to p53 negative ones. Higher mean values were found at recurrence even in patients who showed no change on histology.

Alteration of p53 gene being an early detectable change in tumorigenesis and progression, p53 expression may indicate an increased susceptibility to an earlier recurrence.

### 061P. Expression of Glucose Transporter 5 in Human Gliomas: Characteristics of Microglial Phenotype

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Our previous studies indicated that glucose transporter 5 (GLUT5) is a microglial marker in routine paraffin sections, and is rarely present in monocytes/macrophages of the peripheral organs. We investigated the amounts and activation status of microglia in glioma tissues. The GLUT5 immunostaining was performed in 94 gliomas, together with Ki-M1P, CR3/43, macrophage scavenger receptor-A (MSR-A), GFAP, and MIB-1. The GLUT5-positive microglia with forms of ramified to ameboid were present in all types of astrocytic tumors. In pilocytic astrocytomas, many GLUT5-positive microglia were infiltrated in tumor tissues (up to 52% in total cells), and showed mild hypertrophy and variable expression of CR3/43 and MSR-A. In contrast, glioblastomas contained microglial cells with predominantly hypertrophic and ameboid morphology, and consistently expressed all three markers. A double-labeling study of astrocytic tumors using GLUT5 and MIB-1 antibodies demonstrated the growth fraction of microglia. The GLUT-5 expression on neoplastic glia was not evident except in one case of glioblastoma, where a double-labeling of GLUT5 and GFAP was found on a small number of lipidized, neoplastic cells. The present study indicated that GLUT5 is a useful marker for tumor-associated microglia in glioma tissues, and that pilocytic astrocytomas often have a high proportion of microglial cells with mild activation and proliferative ability.

### 062P. Immunohistochemical Study of Extracellular Matrix in Pediatric Glioblastoma Multiforme and its Correlation with Survival

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*Background*. In the last decades multiple studies have assessed specific factors related to glioblastoma multiforme (GBM) invasion. Surprisingly, in the literature there is a lack of information regarding the tumor cell's interaction with specific extracellular matrix (ECM) components in paediatric GBM. The goal of this study were *i)* to evaluate ECM in paediatric GBM and *ii)* to assess its correlation with survival.

*Methods and results*. ECM was evaluated in 6 cases of pediatric GBM assessing the immunohistochemical expression of tenascin-C (TN-C), laminin (LM), fibronectin (FN), and collagen type IV (C-IV). We used a semiquantitative scale, ranging from not detected (zero) to marked (3), and we correlated the expression of these proteins with survival in each case. Longer survivals were demonstrated in patients with lower scores for ECM. Statistical analysis evidenced a significant correlation between expression of TN-C ($r = -0.9258$; $p < 0.05$), LM ($r = -0.8783$; $p < 0.05$), FN ($r = -0.9258$; $p < 0.05$), and C-IV ($r = -0.8452$; $p < 0.05$) and survival.

*Conclusions*. Although based on a limited number of patients, this study provides additional insights into tumor invasion of pediatric GBM. These informations may offer significant advantages in the management of paediatric GBM leading to improved prognosis and cure.

### 063P. Applicability of Novel Markers as Correlates of Tumor Biology in Pediatric Malignant Astrocytomas

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*Background*. The basis for widely differing outcomes in pediatric malignant astrocytomas has largely been enigmatic, thus hampering efforts at therapeutic stratification.

*Methods*. To evaluate the utility of markers of tumor biology as means for refining prognostic assessment, we retrospectively analyzed 62 pediatric malignant astrocytomas (range 9 months-15 years) of the CNS, diagnosed between 1995-2001.The expression of p53,epidermal growth factor receptor(EGFR), Bcl-2 and retinoblastoma(pRb) proteins was analyzed by immunohistochemistry and results were compared with clinical profile,MIB-1 index and patient survival.

*Results*. Most of tumors were located supratentorially (74%). Histologically 87% were glioblastomas(GBM) and 13% were anaplastic astrocytomas(AA). Amongst GBMs,intense p53 immunoreactivity was noted in 55.6% of cases,predominantly in cerebral lobar tumors. Duration of symptoms was shortest in this group (mean:2 months) which further correlated with the highest MIB-1 index ($17.20 \pm 3.21\%$), compared to p53 negative tumors (p0.003). EGFR and Bcl-2 overexpression and absent pRb was observed in 25%, 29% and 7.4% cases respectively. p53 and lack of pRb was maximally noted in tumors where patients had very early recurrence ($n = 6$) or died of disease ($n = 3$). p53 was absent in all AAs. Only one tumor lacked pRb and this patient had the longest survival (>3 years). Expression of markers was negligible in infratentorial tumors.

*Conclusion*. Our results suggest that supratentorial pediatric denovo GBMs show high p53 expression proving to be a strong predictor of outcome along with MIB-1 index. Lack of pRb though minimal, signifies worse prognosis in GBMs unlike in AAs. Infratentorial tumors have different pathogenetic pathways. Further molecular characterization would provide new insights for refining therapeutic decision-making.

### 064P. A Tanycytic Differentiation of Chordoid Glioma of the Third Ventricle

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A chordoid glioma in the third ventricle was studied immunohistochemically and ultrastructurally special attention was paid to the histogenesis in relation to the pathological appearance and unique anatomic location of this tumor.

A 65-year-old woman presented with a 2-month history of headache. MR imaging demonstrated a well-circumscribed enhanced mass in the anterior part of the third ventricle, measuring 2.5 cm in diameter. Solid tumor was subtotally resected. CT scans two years postoperatively did not detect any sign of tumor recurrence. Light microscopically, the tumor consisted of clusters and strands of epithelioid cells and elongated bipolar cells in a mucinous matrix. Immunohistochemical findings were similar to those reported previously, including positive reaction for GFAP, vimentin, and CD34. Furthermore, immunopositivity for laminin, collagen type IV and E-cadherin was demonstrated. Ultrastructurally, microvilli were frequently seen, and three types of abnormal cilia were rarely observed. Basement membrane around the tumor cells and microvessels was extensive. Poorly to moderately developed intermediate (adherent) junctions were frequently seen. Resemblance of these ultrastructural features of the tumor to embryonic tanycytes suggests the tanycytic differentiation of chordoid glioma. Neuroradiologically, all of previously reported cases of chordoid gliomas seemed to arise in the anterior part of the third ventricular floor. This region includes lamina terminalis, infundibular recess and median eminence, which corresponds to a tanycyte rich area. These findings suggest the tanycytic origin of chordoid glioma.

## WORKSHOPS

### New Neuropathology of Parkinson's Disease and Related Synucleinopathies

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Modern neuropathologic methods reveal abnormalities in tau and synuclein in most Parkinsonian disorders, which is the basis of a molecular classification of Parkinson's disease (PD). Synuclein inclusions characterize Lewy body disease (LBD) and multiple system atrophy (MSA), while tau pathology is found in progressive supranuclear palsy (PSP), corticobasal degeneration (CBD) and post-encephalitic Parkinsonism (PEP). Neuronal loss in the substantia nigra causes the extrapyramidal features, but pathology is not limited to the nigra and all disorders affect multiple systems. While Lewy bodies (LBs) are well known in PD, neuritic pathology and to a lesser extent glial lesions are increasingly recognized. Extranigral pathology, including olfactory and amgdala lesions, are the focus of recent efforts to stage PD. Glial cytoplasmic inclusions are found in MSA, but immunohistochemistry also shows neuronal cytoplasmic and nuclear inclusions. While tau related neuronal and glial lesions characterize PSP, CBD and PEP, tau has also recently been implicated in PD. Genetic studies suggest that tau may be a risk factor and tau immunoreactivity is detected in many, but not all LBs. Less often, neither tau nor synuclein inclusions are detected in Parkinsonian brains such as in frontotemporal dementia with Parkinsonism and in autosomal recessive juvenile Parkinsonism (ARJP) due to mutations in Parkin, a ubiquitin ligase. As ARJP and ubiquitin immunohistochemistry indicate, abnormalities of the ubiquitin-proteasomal system are increasingly recognized as important in the pathogenesis of Parkinsonism.

### Convergence of Tau and Alpha-Synuclein Pathology in Neurodegenerative Disease

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Tauopathies and synucleinopathies define 2 groups of neurodegenerative disease characterized by filamentous inclusions composed of tau or alpha-synuclein (AS), respectively. However, there is increasing evidence that AS and tau pathology often co-occur. Parkinson's disease (PD) is defined by the presence of AS pathology in the form of Lewy bodies in the brainstem. In PD patients, tau pathology was consistently detected in limbic regions as well as the brainstem. Biochemical analysis revealed accumulation of abundant insoluble tau in affected brain regions. Moreover, distinct tau isoforms accumulated in limbic regions and brainstem despite no change in tau expression. To investigate possible mechanisms for this convergent pathology, we analyzed a transgenic mouse model of synucleinopathy generated by expressing human AS containing the familial PD mutation A53T. These mice develop motor impairments that coincide with abundant AS inclusions. A subset of these mice accumulated tau-positive pathology in affected brain regions. Similar to the human disorders, pathological inclusions were comprised of tau alone, AS alone or both proteins. These data suggest that AS and tau may interact through unidentified mechanisms. We tested the hypothesis that tau and AS promote the fibrillization of each other by co-incubating these proteins in vitro. We demonstrated that AS induces fibrillization of tau and co-incubation of tau and AS synergistically promotes fibrillization of both proteins. These results suggest that interactions between amyloidogenic proteins drive protein fibrillization and aggregation in human neurodegenerative diseases.

### Regulation of α-Synuclein Fibrillization

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Filamentous inclusions of α-synuclein protein are hallmarks of neurodegenerative diseases collectively known as synucleinopathies. To determine how α-synuclein fibrillizes into filaments under various conditions, we performed in vitro studies using recombinant wild-type and mutant α-synuclein. Our results suggest that α-synuclein readily fibrillize in vitro and that the A53T mutant α-synuclein found in patients with familiar Parkinsons disease (PD) fibrillize faster that wild type α-synuclein. We next investigate the contibution of N- and C-termini on α-synuclein fibrillization and found that the C-terminus of α-synuclein inhibit fibrillization. To study the role of oxidative and nitrative stress in α-synuclein fibrillization, we used a molecular and biochemical approach to assess their effects on α-synuclein fibril formation and fibril stabilization. The results of our experiments demonstrate that nitrative and/or oxidative stress utilizes distinct mechanisms in α-synuclein protein modifications that can influence the formation of stable α-synuclein fibrils.

### Mouse Models of Neuronal Alpha-Synucleinopathies: A Brain Amyloidosis

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Alpha-synucleinopathies are human neurodegenerative disorders that are caused by the neurotoxic effects resulting from the accumulation of alpha-synuclein (AS) in amyloid deposits in neurons and glia. This presentation reviews AS transgenic (TG) mouse models of human alpha-synucleinopathies and in particular lines of TG mice expressing A53T human AS in CNS neurons that developed a lethal motor impairment associated with the age-dependent appearance of intracytoplasmic neuronal AS amyloid inclusions similar to human Lewy bodies, Lewy neurites and neuroaxonal spheroids in association with axonal degeneration. Like their human counterparts, these AS amyloid lesions were AS positive and they also stained with amyloid dyes such as Thioflavin-S and silver methods. The inclusions also were ubiquitinated and contained neurofilament proteins, while AS isolated from Tg mouse brains was insoluble and immuno-electron microscopy demonstrated ~10 nm fibrils in the AS amyloid inclusions. Remarkably, some of the A53T AS TG mice also showed tau inclusions similar to the convergence of tau and AS amyloids in human disease. Thus, these TG recapitulate key features of human alpha-synucleinopathies including concomitant accumulations of tau pathologies.

## PLATFORM PRESENTATIONS

### 065. Neuropathology of Familial Cortical Lewy Body Disease

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*Background*. Few reports of families with inherited dementia with Lewy bodies have been identified in the literature, with limited descriptions of their pathology. We identified 5 cases with familial cortical Lewy body disease, and describe their pathology.

*Methods*. The families of dementia patients with neuropathologically confirmed Lewy body disease were approached to identify other relatives with Parkinson's disease (PD) and/or dementia. Five families were identified that had 3 or more affected individuals consistent with a dominant inheritance pattern. The brain tissue was examined to determine the quantity and extent of pathology.

*Results*. The index cases consisted of 3 men and 2 women with age at death ranging from 45 to 84 years. Two families had predominant dementia and the pathology of the cases was numerous plaques and neurofibrillary tangles satisfying criteria for Alzheimer's disease, in addition to numerous cortical Lewy bodies. One family had a predominantly PD clinical presentation, with neuropathology showing frequent plaques and cortical Lewy bodies but no evidence of neurofibrillary tangles. The pathology of the index case in 2 families where family members had mixed dementia and parkinsonism, showed minimal plaque and infrequent neurofibrillary tangles.

*Conclusions*. The neuropathological presentation of familial cortical Lewy body disease is highly variabile, where frequent cortical Lewy bodies may or may not be accompanied by plaque or neurofibrillary tangles.

### 066. α-Synuclein Pathology in Parkinson and Alzheimer Diseases

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*Background*. Recent data suggest a staging of α-synuclein (AS) pathology wth ascending progression in Parkinson's disease (PD) and frequent involvement of Alzheimer's disease (AD) brain.

*Material and methods*. The incidence and distribution of AS-positive inclusions (Lewy bodies and neurites) was examined in 197 brains of elderly subjects (51 cases of clinical PD, 104 autopsy-proven AD, 22 dementia with Lewy bodies (DLB), 20 age-matched controls). The olfactory system was not studied.

*Results*. In PD AS-positive lesions involved medullary and pontine nuclei, locus ceruleus, substantia nigra pars compacta (100%), nucleus basalis of Meynert (NBM) (78%), amygdala (41%), allocortex (57%), cingulate cortex (34%), and isocortex (26%) corresponding to pathology stages 4-6 (Braak et al, 2003). All DLB brains, except for one case without medullary involvement, irrespective of coexisting AD lesions, showed AS pathology equivalent to PD stages 5 or 6. Controls and 50% of AD brains were AS negative, 50% revealed AS-lesions: nigra (33%), locus coeruleus (23%), amygdala (16.4%), NBM and allocortex (14%), oblongata (11%), cingulate area (9%), isocortex (0). Nine cases of incidental Lewy body disease (preclinical PD) corresponded to stage 3 or 4; 5 AD brains (2 with parkinsonism) had AS lesions in midbrain, NBM and allocortex without medullary involvement suggesting deviation of the proposed "stereotypic" expansion pattern of AS pathology.

*Conclusion*. The present data largely confirm the staging of AS-pathology in PD proposed by Braak et al (2003) with rare preservation of the medulla and the frequent occurence of AS-lesions in AD, but the pathogenesis and impact of AS-pathology in PD and AD need further elucidation.

### 067. α-Synuclein Accumulates in Purkinje Cells in Lewy Body Disease but not in Multiple System Atrophy

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*Background*. α-Synuclein has important roles in the pathogenesis of Parkinson's disease (PD), dementia with Lewy bodies (DLB) and multiple system atrophy (MSA). Purkinje cell depletion is present in MSA. By contrast, cerebellar pathology has not been demonstrated unequivocally in either PD or DLB. Recent studies showed that LB-type degeneration in PD and DLB is more widespread than previously recognized.

*Methods*. To determine whether Purkinje cells might be involved in α-synuclein pathology, we performed immunohistochemical examinations of the cerebella of patients with PD, DLB, MSA, various tauopathies, and control subjects.

*Results*. Although no abnormal accumulation of α-synuclein was noted in the Purkinje cell somata, numerous α-synuclein-positive round inclusions were found in the white matter in all the patients with PD and DLB. Immunohistochemical and ultrastructural examinations revealed that the majority of these inclusions was located in the Purkinje cell axons and consisted of granulo-filamentous structures. No such inclusions were observed in MSA, tauopathies or controls.

*Conclusion*. These findings indicate that Purkinje cells are also the victims of α-synuclein pathology in PD and DLB, but not in MSA.

### 068. Striatal α-Synuclein (ASN) Burden Correlates with Disease Duration in Parkinson's Disease (PD), but not Dementia with Lewy Bodies (DLB)

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*Background*. Extranigral pathology is increasingly recognized in PD, especially with sensitive methods to detect ASN. The clinical significance is not always clear.

*Methods*. We studied striatal pathology with ASN immunohistochemistry in age- and sex-matched cases of PD (N = 10), PD with dementia (PDD; N = 7), all of whom had dementia onset >2 years after onset of parkinsonism, and DLB (N = 20). The PD cases

included 3 brainstem (BLBD), 6 transitional (TLBD) and 1 diffuse Lewy body disease (DLBD); PDD included 2 BLBD and 5 DLBD; and DLB included 8 TLBD and 12 DLBD. Image analysis was used to measure the density of ASN-immunoreactive neurites in the putamen with an antibody to ASN-phospho-serine 129.

*Results*. ASN burden was significantly less in PD (0.3%) than in PDD (0.6%) and DLB (0.5%), while PDD and DLB did not differ. The ASN burden correlated with disease duration for PD and PDD (r=0.6), but not DLB. Considered by LBD subtype, striatal ASN burden was significantly greater in DLBD than in BLBD, but similar to TLBD. Discussion: These results show similarity in extranigral ASN pathology in DLB and PDD even though the clinical syndromes differ. Striatal pathology in PDD appears to be a progressive process is detected only in later stages of disease, while in DLB striatal pathology is earlier and not related to disease course. Alternatively, widespread ASN pathology may be present from the beginning in DLB, suggesting a different pathogenesis from PD and PDD.

### 069. Mutation Spectrum of Park2 Gene in Parkinson's Disease Patients from Russia

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Parkinson's disease (PD) is common neurodegenerative disorder, characterized clinically by a combination of motor symptoms (akinesia, rest-tremor, rigidity and disturbance of postural reflexes). Pathologically, there is a rather selective degeneration of dopaminergic neurons of the substantia nigra, leading to a deficiency of dopamine in the striatal projections areas of these neurons. Mutations in the Park2 gene have recently been identified in patients with early onset autosomal recessive Parkinson's disease. We studied 31 families from Russia in which at least one of the affected family members was affected before the age of 45 years and also 38 patients with sporadic idiopathic PD with onset after the age of 50 years. All subjects were screened for mutations in the Park2 gene with use of semiquantitative PCR assay that simultaneously amplified several exons. Among the families with early-onset PD 35% had deletions in the Park2 gene. Among the patients with sporadic idiopathic PD, deletions were detected in 5% of patients. Several single exon deletions were revealed. The most frequent is deletion of 6th exon (22%). Deletion of 12th exon is observed for the first time. It is revealed in patients with sporadic idiopathic PD with onset after the age 50 years. This study confirm that Park2 gene mutations are not only major causes of early-onset autosomal recessive families PD but also could be responsible for later-onset idiopathic form of disease including sporadic cases.

### 070. Down Regulation of Nitrergic Transmission in Rat Striatum After Dopamine Deafferentation

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Although nitric oxide (NO) may be involved in the pathogenesis of basal ganglia degeneration, it can play a crucial role in the mechanisms of neuronal plasticity in the striatum in rodent models of hemiparkinsonism. After lesion of substantia nigra, nNOS activity decreases by 50% in rat striatum. However, it is unclear whether a down regulation of NO/cGMP synthetic pathway comes about from such reduced nNOS activity, or whether this can be compensated by co-occurrence of low levels of cyclic nucleotide phosphodiesterases (PDEs), enzymes degrading NO-induced cGMP and dopamine-induced cAMP. We evaluated levels of cGMP and cAMP, and expression of nNOS and PDE1B isoform in rat striatum homogenates, in which dopaminergic pathway was unilaterally lesioned two months before. In addition, using cytochemical procedures on brain sections we characterized the distribution of nNOS and PDE1B within deafferented striatal neurons. NOS positive intrastriatal nerve fibers showed specific plastic changes with a highly significant reduction in density, while a large population of medium-sized striatal neurons showed an evident increase of PDE1B immunoreactivity. Accordingly, nNOS expression and cGMP accumulation were both markedly reduced. Moreover, while cAMP levels were markedly increased, PDE1B activity and PDE1B mRNA expression were significantly up-regulated. These changes all can concur to down regulate the nitrergic transmission in dopamine-deafferented striatal neurons, showing new aspects of neuronal plasticity in experimental hemiparkinsonism.

## POSTERS

### 071P. An Autopsy Case of Juvenile Onset Parkinson's Disease with α-Synuclein Positive Oligodendrocytic Coil-Like Inclusions Widely in the Reticular Formation System

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A 62-year-old woman was suffered from l-dopa responsive parkinsonism since 31-years-old without diurnal fluctuation. Five years before death, she showed disturbance of attention. Impairment of arousal and vigilance were remarkable, so she often falled in sleep during eating. She was found as dead in front of the television at her home in the early morning. Neuropathological examination revealed that decrease of pigmented neurons were severe in the substantia nigra, and moderate in the leucus cerureus and dorsal vagal nucleus with a little gliosis. Many Lewy bodies were observed in the raphe, dorsal vagal nucleus and Meynert nucleus without senile palques and neurofibrillary tangles. Modifeid Gallyas-Braak staining disclosed oligodendrocytic coil-like inclusions. The inclusions were positive to α-synuclein and patially to ubiquitin and negative to tau, immunohistochemically. They were distributed widely in the reticular formation system; reticlular formation in the brainstem, thalamic intralaminar nuclei and reticular nuclei, spinal intermedial nuclei, cerebral white matter. We think the distribution of the α-synuclein positive oligodendrocytic coil-like inclusions would be associated with impairment of arousal and vigilance.

### 072P. Modified Concentrations of Silver, Cadmium and Nickel in the Erythrocytes and Plasma of Patients with Parkinson's Disease

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Heavy metals are possibly implicated in the pathogenesis of Parkinson's disease. Modification in metals profile may indicate disruption of cellular function at several levels such as storage sites, carrier systems and enzyme activity. The present experiments were designed to analyze concentrations of silver (107Ag), cadmium (114Cd), nickel (60Ni), cobalt (59Co), rubidium (85Rb), strontium (88Sr) and molybdenum (98Mo) in the erythrocytes and plasma of patients with Parkinson's disease using inductively coupled mass spectrometry (ICP-MS) technique. Samples were obtained from 12 Parkinson's patients receiving L-dopa (9 men and 3 women). Elevated levels were observed for 107Ag ($p<0.005$), 114Cd ($p< 0.05$) and 60Ni ($p<0.05$) in the erythrocytes. Concentration of 60Ni was significantly increased ($p<0.01$) in the plasma. Nickel and cadmium ions could interfere with copper and zinc in the synthesis of eumelanin, pheomelanin and oplomelanin. Nickel ions may as well enter substantia nigra if iron concentration is lowered. Nickel also simulates dopachrome oxidoreductase thus decreasing L-dopamine availability. Furthermore catalytic effects of silver, nickel and cadmium possibly lead to the formation of oxygen free radicals that oxidize lipids to lipofuscin and destroy tissues. ICP-MS may be a useful method in estimating elemental biomarkers of blood cells in the Parkinson's patients.

### 073P. Persistent Extracellular Signal Regulated Protein Kinase Activation and Repression of the cyclicAMP Response Element in Parkinsonian Pathogenesis

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*Background*. Cytoplasmic aggregates of phosphorylated extracellular signal regulated protein kinase (ERK) and ribosomal S6 kinase (RSK) are present in pigmented substantia nigra neurons from patients with Parkinson's disease (PD) and dementia with Lewy bodies (DLB). A similar pattern of kinase localization is observed in dopaminergic cell lines treated with the neurotoxin 6-hydroxydopamine (6-OHDA). As p90-RSK links the ERK signaling pathway to the cyclicAMP response element (CRE)-binding protein (CREB) pathway, we hypothesized that altered subcellular localization of these phosphoproteins contributes to 6-OHDA toxicity by suppressing neuroprotective CRE-dependent responses.

*Methods*. ERK, RSK, and CREB phosphorylation and subcellular localization were assessed by immunoblot and immunocytochemical analyses of B65 cells treated with 6-OHDA. B65 cells transfected with a CRE-luciferase reporter plasmid were used to examine effects on CRE transactivation.

*Results*. 6-OHDA elicited increased levels of phospho-ERK, phospho-RSK, and phospho-CREB. Although phospho-RSK was present in both nuclear and cytoplasmic fractions, 6-OHDA-treated cells displayed increases in cytoplasmic CREB, associated with repression of CRE-dependent promoter activity.

*Conclusions*. These studies suggest that cytoplasmic diversion of ERK and CREB signaling pathways may play an important role in Parkinsonian neurodegeneration.

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### 074P. Expression of DJ-1 (PARK7) in Control and Parkinson's Disease Tissue

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*Background*. DJ-1 (PARK7) is the most recent PD gene identified to have mutations causing autosomal recessive PD in two isolated families. The distribution of DJ-1 in human brain tissue is unknown.

*Methods*.We have investigated the distribution of DJ-1 in control and sporadic PD brain by immunohistochemistry (IH), immunoelectron microscopy, western blotting and in-s itu hybridisation. Results: We show that DJ-1 immunoreactivity (DJ-1 IR) is present in high amounts in a proportion of astrocytes in both control and PD brain. DJ-1 IR is present in both the nucleus and cytosol of glial cells. Specificity of glial staining of DJ-1 was demonstrated by preabsorption of the antibody by recombinant DJ-1 or omission of primary antibody. Few neurones expressed low DJ-1 IR. Only occasional Lewy bodies (LBs) and Lewy neurites (LNs), the pathological hall marks of sporadic PD tissue, exhibited DJ-1 IR. We did not find any cortical LBs stained for DJ-1 IR. By western blotting we show that DJ-1 is abundantly expressed in frontal cortex of control and sporadic PD tissue, a region known to form LBs.

*Conclusion*. We conclude that DJ-1 is not an essential component of LBs and is expressed mainly by astrocytes in adult human brain.

### 075P. Pathology of the Ciliary Ganglia in Multiple System Atrophy and Pure Autonomic Failure

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*Backgrounds*. Autonomic disturbance is a main feature of multiple system atrophy (MSA) and pure autonomic failure (PAF). However, little information about the pathology of pupillary autonomic system is available. To delineate the pupil size-related pathology, we studied the ciliary ganglia in patients with MSA and PAF.

*Methods*. Autopsied cases of cerebellar type of MSA (MSA-C), parkinsonian type of MSA (MSA-P) and PAF were used in this study. All were males and the age at exitus was 59, 64 and 74, respectively. Two additional individuals without neurological disorders (73 and 73 year old, both males) were used as control cases. Ciliary ganglion tissue was fixed in 4% buffered formalin, and cut at 10 μm-thick paraffin sections. Sections were stained with hematoxylin-eosin (HE), Kluver-Barrera and Nissl stain, and immunohistochemically with anti-alpha-synuclein monoclonal antibody (LB509, diluted 1:100).

*Results*. In control and MSA-P, there were no abnormal findings in ciliary ganglia. In MSA-C, there was an atrophy and reduction of the ganglion cells. Alpha-synuclein positive Lewy body was found in ganglion cells of PAF.

*Conclusions*. The pathology of ciliary ganglia differed between MSA and PAF. MSA shows simple atrophy of the ganglion cells. PAF is one of the Lewy body type alpha-synucleinopathies targeting the peripheral autonomic nervous system.

### 076P. Immunostaining of Protein Phosphatase 2A in Glial Cytoplasmic Inclusion of Multiple System Atrophy

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*Background*. Protein phosphatase 2A (PP2A) describes an extended family of intracellular protein serine/threonine phosphatases sharing a common catalytic subunit that regulates a variety of cellular functions. Immunohistochemistry with an antibody to PP2A revealed that PP2A is localized mainly in neurons of various regions of rat brain. Recently α-synuclein is reported to be phosphorylated in synucleinopathy lesions of multiple system atrophy (MSA), indicating the importance of phosphorylation in the pathogenesis. Here we have investigated immunostaining of PP2A in glial cytoplasmic inclusions (GCI) of MSA.

*Materials and methods*. Four autopsied brains of MSA were employed. Tissues were fixed with formalin and paraffin-embedded. Sections were immunostained with anti-PP2A antibody (Santa Cruz, sc-6113, x1000) by ABC method. Gallyas staining and immunohistochemistry with an antibody to α-synuclein were also carried out.

*Results*. About half of the GCIs were PP2A-positive. Neither shape of the GCI, nor severity of the degeneration was associated with the immunoreactivity of PP2A.

*Conclusions*. This finding suggests that PP2A expression in the glial cells of MSA brains might be involved in GCI formation.

### 077P. An Autopsy Case of Striatonigral Degeneration with Numerous Neuronal Cytoplasmic Inclusions in the Brainstem and Neuronal Nuclear Inclusions in the Cerebral Cortex

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The distribution and intracellular localization of α-synuclein may provide new perspectives to understand striatonigral degeneration (SND), a subgroup of multiple system atrophy (MSA). A 73-year-old female with L-Dopa responsive parkinsonism died after a 6-year clinical course. Cerebellar ataxia and autonomic failure, such as orthstatic hypotention, were not noticed throughout the illness. Radioimaging revealed a hyposignal region in the lateral putamen and atrophy of the pons and cerebellum. Neuropathological examinations confirmed the presence of SND and olivopontocerebellar atrophy. Numerous α-synuclein positive NCI were observed in the pontine neurons. Neurons in the cerebral cortex were well preserved, but NNI was widely observed. The present case suggests, *i)* there is a group of MSA patients with numerous NCI in the brainstem neurons, *ii)* the intracellular metabolism of α-synuclein is different in cortical and brainstem, and *iii)* NCI do not necessarily lead to neuronal death because numerous NCI containing pontine neurons were observed during the 6-year clinical course.

### 078P. α-Synuclein Expression in Rat Brain Following 6-OHDA Nigrostriatal Lesions

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α-Synuclein is a member of a relatively new family of proteins considered to be involved in pathogenesis of PD and other neurodegenerative diseases. Its filamentous aggregates are found as principal constituents of Lewy bodies and some others pathological intraneuronal deposits. Due to the localization of α-synuclein in presynaptic terminals, its role in synaptic function and plasticity was postulated as well. 6-OHDA rat model of PD is characterized by acute loss of dopaminergic neurons, but specific α-synuclein aggregates were not reported. Therefore, this model could point to possible role of α-synuclein in synaptic plasticity after chemical injury given that some compensatory sprouting of dopaminergic neurons exists after period of extensive neuronal death.

Changes in α-synucelin expression were followed in striatum and mesencephalon, as well in the structures anatomically or functionally related to the nigrostiatal system, 40 days after 4-site unilateral intrastriatal 6-OHDA injections. Using Western blot analysis, we detected increase in α-synuclein expression in ipsilateral striatum, mesencephalon and cerebellum, comparing to sham operated controls. Observed increase was the most prominent in cerebellum. In ipsilateral cortex, α-synuclein protein expression remained unaffected. Synaptophysin, used as marker of synaptic density, followed different pattern of expression. High increase in protein level was observed in ipsilateral cortex and striatum only.

In conclusion, our results indicate the involvement of α-synuclein in synaptic plasticity in striatum, mesencephalon and cerebellum following chemical injury.

### 079P. Familial Dementia with Lewy Bodies

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We report a rare familial case of dementia with Lewy bodies (DLB). Three patients, all siblings whose parents were first cousins, were clinically diagnosed as having DLB. Two deceased siblings were clinically characterized by parkinsonism of youthful onset followed by dementia after a long disease duration. The surviving sister shows slowly progressive dementia of early onset. At autopsy, neuropathological examinations of both the proband and his brother showed severe changes in the hippocampus CA2-3, perirhinal cortex, amygdala, and subcortical nuclei such as nucleus basalis of Meynert, substantia nigra, and hypothalamus. Both patients showed no senile plaques and very few neurofibrillary tangles (NFTs). From the viewpoint of Lewy body (LB) distributions, those observed in the proband's brother were of the limbic (transitional) type, whereas those in the proband were of the neocortical type in spite of his having a shorter disease duration and milder clinical symptoms than his brother. We considered that neuronal loss of the perirhinal cortex and hippocampal CA2-3 would be an essential process in this familial DLB brain.

### 080P. Accumulation of 14-3-3 Proteins in Lewy Bodies and Glial Cytoplasmic Inclusions

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*Objective*. α-Synuclein, a major component of Lewy bodies (LBs) and glial cytoplasmic inclusions (GCIs), has been reported to interact with 14-3-3 proteins. To elucidate the role of 14-3-3 in LBs and GCIs, we performed immunohistochemical studies on 14-3-3 in autopsied brains from patients with α-synuclein-related diseases.

*Methods*. We selected postmortem brains from 7 patients with Parkinson's disease (PD), 3 patients with diffuse Lewy body disease (DLBD), 15 patients with multiple system atrophy (MSA), and 7 normal control subjects. Paraffin-embedded sections from all cases were immunostaind using a specific anti-14-3-3 antibody.

*Results*. In both normal and disease cases, 14-3-3 immunoreactivity was mainly observed in the neuronal somata and processes. In addition, brainstem-typed and cortical LBs were intensely immunostained in the PD and DLBD cases, and numerous GCIs were densely immunolabeled in the MSA cases. Strongly immunopositive neuronal cytoplasmic inclusions (NCIs) were occasionally found in the pontine nucleus from the MS cases, and some dystrophic neurites were also immunoreactive for 14-3-3 in all three types of disorders.

*Conclusions*. Our results suggest that an aberrant accumulation of 14-3-3 proteins may occur in brains affected by PD, DLBD, and MSA, and that 14-3-3 may play an important role in the pathogenesis of synucleinopathies.

### 081P. Triple Cerebral Amyloidosis in Human Niemann-Pick Type C Disease

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Tauopathy is a feature of Niemann-Pick disease type C (NPC). We have reported an accumulation of beta-amyloid (Aβ) in NPC patients with apoE4 homozygosity (Saito Y et al: Ann Neurol 2002). Now we report that phosphorylated alpha-synuclein (psyn) also accumulates in the brain with NPC. The central nervous system from twelve NPC cases were examined immunohistochemically. Ages of the patients ranged from 9 months to 55 years. Antibodies raised against psyn (psyn#64), phosphorylated tau (ptau, AT8), Abeta (4G8) and apoE4 were employed. Immunohistochemistry with psyn#64 detected LB in 2 cases, in both of whom the apoE4-epitope was seen but deposition of Aβ was detected in one case only. Eight of the remaining 10 cases showed diffuse but strong perikaryal psyn-immunoreactivity in the swollen storage neurons including 2 cases with apoE4, 4. The psyn-immunoreactivity was most prominent in substantia nigra, an area with most severe tauopathy. Co-localization of psyn- and ptau-epitope was frequent in neuronal structure. No psyn-immunoreactivity was observed in 2 youngest cases, 9 months and 30 months of age. This is the first demonstration of alpha-synucleinopathy in human NPC. NPC with apoE4 homozygosity could cause triple amyloidosis in the central nervous system.

## WORKSHOPS

### The Nerve Microenvironment and Anatomy of Nerve Injury

Powell HC; Myers RR

The microenvironment of peripheral nerve is finely regulated by theselective permeability of the blood-nerve-barrier (BNB) an interface formed by capillary endothelium and the innermost layers of the perineurium, a semi-elastic sheath that is resistant to expansion such as endoneurialedema. Nerve edema is a common response to nerve injury, for example in Wallerian degeneration the endoneurial environment is transformed by mast cell activity, altered permeability, macrophage immigration and nerve fiber disintegration. A critical factor in considering the nerve microenvironment is the architecture of the vasa nervorum, a microvascular plexus running in a longitudinal orientation along the nerve axis and giving off branches that traverse the perineurial sheath. These transperineurial vessels are vulnerable to the "pinch" effect of increased pressure due either to endoneurial edema of external compression. Endoneurial vessels are lined by endothelial cells forming tight junctions in the extraganglionic endoneurium. Devoid of lymphatics the endoneurial interstitium is permeated by endoneurial fluid under a constant positive pressure. Endoneurial fluid pressure (EFP) is elevated during Wallerian degeneration, reaching its peak seven days after proximal crush injury. A slower progression of elevated EFP occurs in lead poisoning after endothelial cells show evidence of intoxication, followed inevitably by Schwann cell injury and demyelination. By contrast, experimental allergic neuritis involves edema due to interendothelial cell separation associated with immigration of lymphocytes and macrophages into the endoneurial space, and local anesthetics applied to the nerve sheath increase its permeability and elevate EFP. Other toxins such as hexachlorophene and galactose increase EFP through effects on the myelin sheath or schwann cell, without apparent ingress of macromolecules. Elevated EFP may also reduce nerve blood flow with adverse consequences for the nerve fiber and the microenvironment.

### Experimental Diabetic Autonomic Neuropathy: New Animal Models and Hypotheses

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Neuroaxonal dystrophy (NAD), a distinctive axonopathy involving distal axons and synapses, represents the neuropathologic hallmark of diabetic sympathetic autonomic neuropathy in man and several insulinopenic experimental rodent models. The streptozotocin (STZ)-diabetic and BBW-diabetic rat develop marked hyperglycemia and concomitant deficiency in both circulating insulin and IGF-I. These animals reproducibly develop NAD in nerve terminals in the prevertebral sympathetic ganglia and the distal portions of noradrenergic ileal mesenteric nerves. The Zucker diabetic fatty (ZDF) rat and the BBZ diabetic rat, both animal models of type 2 diabetes, also develop severe hyperglycemia comparable to that in the STZ-diabetic rat models, although in the presence of hyperinsulinemia and, at least for the ZDF rat, normal levels of circulating IGF-I. NAD did not develop in 6 to 8 month diabetic ZDF and BBZ rat sympathetic ganglia and ileal mesenteric nerves as assessed by quantitative ultrastructural techniques. Ongoing investigation of several mouse models also provides evidence that hyperglycemia alone is not sufficient to cause sympathetic NAD. Previous studies of STZ-diabetic rat have shown that administration of exogenous IGF-I is capable of normalizing established dystrophic axonopathy in the absence of an effect on plasma glucose and HbA1c. These data argue very strongly that hyperglycemia is not the critical and sufficient element in the pathogenesis of diabetes-induced NAD, rather that it may be the loss of trophic support, most likely of IGF-I or insulin, that causes NAD.

### Neuropathology of Diabetic Neuropathy. Lessons from Animal Models and Patients

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The cause and treatment of human diabetic neuropathy remain to be defined. Furthermore, the basic pathology of human diabetic neuropathy at different stages of the disease is not clear and the relationship of this to measures of neuropathic severity is not established. The role of apoptotic cell death in Schwann cells and in pericytes and endothelial cells of the endoneurial capillaries will be defined using detailed electronmicroscopic morphometry. A large body of evidence implicates microangiopathy in the pathogenesis of this condition, this will be explored in detail drawing upon studies in diabetic patients at various stages of neuropathy and in a number of animal models including STZ-rat, diabetic minipig, diabetic baboon and eNOS knockout mice with and without diabetes. Pathogenetic mechanisms of painful nerve damage will be assessed in rarer and more troublesome presentations such as diabetic amyotrophy and insulin neuritis. Mechanisms of painful neuropathy will be explored in functional and immunohistological studies in the skin of diabetic patients with and without painful neuropathy.

### The Molecular Basis for the Differences Between Type 1 and Type 2 Diabetic Neuropathy

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Traditionally, diabetic polyneuropathy (DPN) was believed to be the same in types 1 and 2 diabetes and was caused by hyperglycemia. It is evident that DPN is more severe and progresses more rapidly in type 1 diabetes. We have suggested that this is due to insulin/C-peptide deficiencies. Under experimental conditions, type 2 DPN shows milder functional deficits, normal regeneration and lacks the nodal changes characterizing human and experimental type 1 DPN.C-peptide has insulinomimetic effects, but no glucose lowering action. We have compared type 1 BB/Wor-rats with type 1 C-peptide replaced rats and isohyperglycemic and normo-C-peptidemic type 2 BB/Z-rats, to dissect the contributions of hyperglycemia versus insulin/C-peptide deficiency to DPN. C-peptide replacement in type 1 DPN ameliorates partially the nerve conduction defect via correction of neural Na/K-ATPase, endoneurial blood flow and NO. It normalizes nerve fiber regeneration by correcting early immediate gene responses, the expression of IGF-1 and its receptor and that of tubulin and neurofilaments. It prevents the nodal/paranodal degenerative changes by normalizing key molecular elements such as caspr, contactin and the Na+-chan-

nel b-subunit. These effects may underlie the beneficial effects of C-peptide on DPN and autonomic neuropathy in type 1 patients. In conclusion, type 1 DPN hyperglycemia plays only a partial pathogenetic role and insulin/C-peptide deficiency plays an equally important role and explains the more severe DPN in type 1 diabetes.

## PLATFORM PRESENTATIONS

### 082. Vascular Lesions in Multifocal Diabetic Neuropathy

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In type 2 diabetic patients, multifocal sensory-motor neuropathies (MSMN) occur less frequently than distal symmetrical sensory neuropathies (DSSN). Usually, the peripheral nerve biopsy shows symmetrical axono-myelinic lesions, predominantly in small myelinated fibres associated with thickening of capillary walls, in patients with DSSN. Asymmetrical axonal lesions between fascicules found in the nerve specimens of patients with MSMN, are suggestive of an ischemic process. In a series of diabetic patients with recent mononeuritis multiplex, bilateral proximal neuropathy or asynchrony distal sensory-motor neuropathy, we lead particular attention to epineurial, perineurial and endoneurial vessels. Axonal degeneration was found in more than 35% of teased fibres and less than 10% of nerve fibres showed demyelination and remyelination. Most nerve biopsy showed typical diabetic microangiopathy similar to DSSN. Endoneurial and perineurial necrotizing vasculitis was found in several patients. Massive endoneurial bleeding was present in a few nerve specimens. Extravasations of red blood cells were observed in many patients and ferric iron deposition in others, giving evidence of previous bleeding. Most nerve specimens had perivascular mononuclear cell infiltrates. Vascular lesions were sometimes found in the muscular biopsy. We suggest that involvement of small arteries and precapillary blood vessels associated with inflammation may be responsible for type 2 multifocal diabetic neuropathies.

### 083. An In Vitro Model of Axonal Damage Following Demyelination

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We used dorsal root ganglia (DRG) cultures from a transgenic (tg) rat model of CMT1A to determine whether it is possible to induce an axonopathy in long term (three months) tg DRG cultures. chracterized by dys-demyelinated internodes. Axonal and myelin structure has been studied by light, electron microscopy (EM) and confocal microscopy. Axonal diameter, periaxonal area and neurofilaments (NF) density were also measured by EM. Phosphorylation of NF was evaluated by western blot analysis. In tg cultures we found peculiar abnormalities of the myelin sheath, which showed a "bead-like" appearance. EM examination showed uncompacted myelin and smaller axons with increased density of NF, in all the tg cultures. Morphometric evaluation demonstrated a significant reduction of axon diameter and an increase of periaxonal area in tg cultures compared to normal ones, together with a higher density of NF. Western blot analysis showed an increased percentage of non-phosphrylated neurofilaments compared to the phosphorylated ones in both homozygous and hemizygous tg cultures. Finally, confocal microscopy demonstrated that, in tg cultures, MAG shows focal accumulation along the internodes. In conclusion, we present an in vitro model of axonal damage secondary to a process of dys-demyeliantion, due to overexpression of PMP22.

### 084. Fine Structural Hallmarks in Molecular Genetically Identified Hereditary Peripheral Neuropathy Type CMT2E (NEFL), CMT4A (GDAP1), CMT4F (Periaxin), Tangier disease, and HMSN L (NDRG1)

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*Background*. Meanwhile 22 genes and about 30 chromosomal loci have been identified as being involved in causing hereditary motor and sensory peripheral neuropathies (HMSN).

*Objectives*. There are only rare nerve biopsies available in recently detected, molecular genetically identified, hereditary peripheral neuropathies.

*Methods*. From a large series of sural nerve biopsies, we used DNA extracted from paraffin-embedded material of selected cases (or from blood specimens in cooperation with others) to identify mutations in inherited peripheral neuropathies. Novel or well known mutations were detected in the neurofilament light gene (NEFL) causing Charcot-Marie-Tooth neuropathy type CMT2E; periaxin (PRX) causing CMT4F; the ganglioside-induced differentiation-associated protein 1 gene (GDAP1) causing CMT4A; the N-myc downstream regulated 1 (NDGR1) causing HMSN Lom; and the ABC1 gene causing Tangier disease.

*Results*. The predominant fine structural features of the neuropathies mentioned will be presented, especially those in periaxin neuropathy, HMSN L, in a syringomyelic variant of Tangier disease, and in "CMT2E" and "CMT4A."

*Conclusions*. The variability of structural changes in the small number of sural nerves studied thus far in molecular genetically defined inherited peripheral neuropathies correlates with the remarkably large spectrum of clinical features. A new HMSN classification is needed.

### 085. Amyloid Neuropathy: a Retrospective Study of 32 Peripheral Nerve Biopsies

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*Background*. We performed a retrospective study of 32 peripheral nerve biopsies (PNB) with amyloid deposits.

*Methods*. In every case, lesions were studied on paraffin-embedded fragments (PEF) and by ultrastructural examination (USE). Direct immunofluorescence with anti-light chain sera was performed in 16 cases, and immunohistochemistry with anti-transthyretin (TTR) in 22.

*Results*. Endoneurial amyloid deposits were easily identified on routine PEF in 24 cases, after Congo red or thioflavine staining in 3, and by USE in 5. PNB disclosed amyloidosis in 16 "sporadic" cases. A dramatic myelinated nerve fiber loss was evidenced in 31 cases (2970-77/mm$^2$), predominating on small fibers in only 4 cases. Amyloid deposits were marked by anti-TTR in the endoneurium in 13 cases and around muscle fibers in 5 others. A mutation on the TTR gene was present in 9 of these 18 cases and in 3 others. In 6 patients with a monoclonal gammopathy, lambda or kappa light chain was evidenced in endoneurial amyloid

deposits. Another patient with an IgG k MG presented no light chain in amyloid deposits but TTR-positive deposits around muscle fibers; afterwards a TTR Met 30 mutation was evidenced. Four cases are still under investigation.

*Conclusion*. In 16 "sporadic" cases amyloidosis was disclosed by PNB and 13 corresponded to TTR pathology.

### 086. A Role for Schwann Cell Dystroglycan in Both Myelination and Nodal Structure

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Dystroglycan is a central component of the dystrophin-glycoprotein complex implicated in the pathogenesis of several neuromuscular diseases. To study its function in peripheral nerves, conditional deletion of Schwann cell dystroglycan was carried out using P0-Cre. This resulted in slowed nerve conduction and nodal changes including reduced sodium channel density and disorganized or blunted Schwann cell microvilli. Additional features of peripheral nerve dysfunction in mutant mice included deficits in rotorod performance and aberrant pain responses. Abnormal myelination was manifested by redundant loops in perinatal mice, then progressively aberrant myelin sheath folding with advancing age. A mild degree of segmental demyelination and axonal degeneration were noted in aging mutant mice. These data indicate that dystroglycan is crucial for both myelination and nodal architecture. Dystroglycan appears to stabilize voltage gated sodium channels at nodes of Ranvier, possibly by mediating trans interactions between Schwann cell microvilli and the nodal axolemma.

## POSTERS

### 087P. Ultrastructure of Peripheral Nerves and the Neuromuscular Junction in Spinal Muscular Atrophy with Respiratory Distress Type 1

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*Background*. Spinal muscular atrophy with respiratory distress type 1 (SMARD1) results from recessive mutations in the gene encoding the immunoglobulin m-binding protein 2 (IGHMBP2) on chromosome 11q13. Affected infants exhibit respiratory distress at age 1 to 6 months. The histological phenotype has not yet been described in patients with confirmed mutations in IGHMBP2. We investigated nerve and muscle specimens from eight SMARD1 patients with genetically confirmed mutations in IGHMBP2.

*Results*. Tibial and sural nerves showed Wallerian degeneration preceded by axonal disintegration. There were no signs of regeneration. Other axonal and myelin abnormalities were present. Atrophic axons contained accumulations of neurofilaments, and myelin sheaths formed tomacula, focal folds, and internal and external loops. All end plates investigated were devoid of an axon but retained the subsynapse and Schwann cell.

*Conclusion*. On the one hand, SMARD1 nerve pathology resembles Wallerian degeneration and cannot be distinguished from "dying-back" types of degeneration in animal models. Compromise of anterograde axonal transport appears to be the causative mechanism. On the other hand, SMARD patients seem to have impaired axon-Schwann cell communication with defects in neuronal adhesion molecules leading to hypo- or hypermyelination.

### 088P. Giant Axons in Charcot-Marie-Tooth Neuropathy Type 2E with Pro22Ser Mutations in the Neurofilament Light Chain Polypeptide

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Neurofilaments (NF) are neuronal-specific intermediate filaments (IF) with important roles in the development and maintenance of axonal structure. The neurofilament light chain polypeptide (NF-L) forms the backbone and initiates assembly of NF. Mutations of NF-L cause the axonal form of Charcot-Marie-Tooth disease (CMT) type 2E (CMT2E) which still lacks detailed pathological descriptions. We identified a Pro22Ser mutation of NF-L in a 5-generation CMT pedigree with three patients examined. Two brothers aged 24 and 18 years manifested the disease in the first decade of life with peroneal atrophy and prominent involvement of the upper limbs; the 47-year-old mother disclosed a less severe involvement of both upper and lower limbs. Neurophysiology in the 3 patients was consistent with an "intermediate" type of neuropathy with features propers of both the axonal and demyelinating neuropathies. The sural nerve biopsy in the mother disclosed a picture of primary axonopathy with axonal atrophy and the occurrence of giant axons, ie, fibers with thin myelin and swollen axons containing an accumulation of disorganized NF. CMT2E must be added to the list of genetical and toxic filamentopathies which share giant axons as their pathological hallmark.

### 089P. Pathology of Hereditary Motor and Sensory Neuropathy Associated with Cerebellar Atrophy (HMSNCA)

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*Background*. HMSNCA is initially reported by us and recently established disease entity as early onset ataxia with ocular motor apraxia and hypoalbuminemia (EAOH) associated with an aprataxin gene mutation. Here we report on 3 autopsy cases of adults with a long clinical course.

*Results*. In the cerebellar cortex, Purkinje cells had disappeared completely with a moderate involvement of the granular cells. The dentate nucleus was preserved. The spinal cord had shrunk to a size less than one-half of normal, and both ascending and descending tracts were severely involved but without active scavenger cells. Neurons of the anterior horn were slightly reduced in number accompanied by gliosis. Large-diameter fibers of the anterior root were slightly decreased in number without obvious axonal changes in the remaining fibers. By contrast, number of large-diameter fibers was

markedly reduced and replaced by fibrosis. Neurons of the dorsal root ganglia were deceased in number with numerous residual nodules. In the sural nerve, a marked loss of myelinated fibers was found with fibrosis. These lesions are pathognomonic in our three cases. Clinically, a moderate mental retardation was reported, but no significant lesions were noted in the brain. Pathognomonic changes responsible for the ocular motor apraxia were not identified in our cases.

### 090P. Demyelinating Peripheral Neuropathy Associated with Familial Hemophagocytic Lymphohistiocytosis. An Immuno-electron Microscopic Study.

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The objective of this presentation is to describe the histological aspects of the peripheral nerve lesions in a case of familial hemophagocytic lymphohistiocytosis (FHL).

An 11 year-old male developed subacute polyradiculoneuropathy, associated with digestive symptoms. Electrophysiological study of peripheral nerve revealed an intense and diffuse demyelinating process. He died several months after the onset of the neuropathic symptoms. Parental consanguinity was present. The laboratory findings including bone marrow smear were consistent with FHL.

Neuromuscular biopsy from the superficial peroneal nerve and peroneal brevis muscle were examined by the following techniques: routine histology, semithin sections, electron microscopy (EM) and immunocytochemistry (light and EM).

The lesions were severe and purely demyelinating. Numerous macrophages entering Schwann cell cytoplasms and destroying myelin sheaths were clearly seen on immuno EM. Most axons were intact. The muscle showed neurogenic atrophy and inflammatory infiltrates of mononuclear cells.

FHL is a rare autosomal recessive disorder in childhood characterised by abnormal immune activation, an uncontrolled inflammatory response with sustained hyperactivation of T-lymphocytes and macrophages.

In 1988, Boutin et al in one similar case described severe axonal lesions, but no cellular infiltration of the parenchyma.

Our case shows that peripheral nerves, in common with other viscera, may be destroyed by the macrophagic infiltration which characterises FHL.

### 091P. Progressive Motor Neuropathy after Syphilis: a Coincidental Association?

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Progressive degeneration of anterior horn cells as well as polyradiculopathy are uncommon neurologic complications of Treponema Pallidum infection even in immunocompetent subjects. Damage of anterior cells in the spinal cord is considered due to chronic meningeal inflammation. Polyradiculopathy might be accounted by altered cell-mediated and humoral immunity. This 61-year-old male when aged 30 was diagnosed having syphilis which was treated with high dose of penicillin. He subsequently failed on follow-up serology. When aged 59, patient experienced cramps, limb weakness, dysphonia, dysarthria, occasional dysphagia.Neurologic examination showed distal limb weakness, more pronounced on the left, moderate wasting, diffuse fasciculations, depressed deep jerks. Sensation was normal. Mental state was normal Negative serology included tests for Borrelia burdogferi and HIV. In serum TPHA was reactive at the dilution of 1:320 and FTA-ABS test was positive. CSF revealed normal protein, cell content and oligoclonal IgG. Electromyography was consistent with acute and chronic neurogenic changes, confirmed by muscle biopsy. Motor conduction velocity was mildly diminished with absent or delayed F-waves. Evoked muscle action potentials in LE were low in amplitude. MRI study revealed multiple unenhancing lesions in the white matter of both hemispheres. Penicillin treatment was repeated followed by IVIg with partial benefit. This case raises the issue of the role of Treponema Pallidum in inducing immune-mediated processes in CNS and PNS, occurring independently from initial infection.

### 092P. Establishment and Characterization of Schwann Cell Lines from Murine Disease Models

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We have previously obtained adult mouse Schwann cell lines either by transfection of SV40 large T antigen gene (MS1) or by spontaneous immortalization (IMS32). More recently, we have established spontaneously immortalized Schwann cell lines from murine models of Niemann-Pick disease type C (NPC), spm/spm (SPMS9), and globoid cell leukodystrophy, twitcher (TwS1). Establishment of such Schwann cell lines derived from murine disease models may greatly facilitate the studies of the cellular mechanisms of their peripheral nervous system lesions in the relevant diseases. In the present study we maintained long-term cultures of Schwann cells derived from dorsal root ganglia and consecutive peripheral nerves of another NPC mice (npcnih/ npcnih, npcnih/+), myelin P0 protein-deficient mice (P0-/-, P0+/-) with their wild-type littermates (P0+/+), and neurofibromatosis type 1 gene (NF1)-deficient mice (Nf1Fcr/+) for 8 to 10 months, and established spontaneously immortalized cell lines from all these animals. These cell lines showed spindle-shaped Schwann cell morphology and distinct Schwann cell phenotypes such as expression of S100, laminin and p75NTR and retained genomic and biochemical abnormalities sufficiently representing the in vivo pathological features of the mutant mice. These cell lines were passaged twice a week and maintained over 10 months without morphological and immunocytochemical alterations. These immortalized Schwann cell lines can be useful in studies of nervous system lesions in these mutant mice and relevant human disorders.

### 093P. C-Peptide Enhances Insulin-Mediated Cell Growth and Protects Against High Glucose Induced Apoptosis in SH-SY5Y Cells

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We have previously reported that C-peptide exerts preventive and therapeutic effects on diabetic neuropathy in type 1 diabetic BB/Wor-rats and prevents duration-dependent hippocampal apoptosis in the same animal model. In the present study, we present data showing that C-peptide stimulates cell proliferation and neurite outgrowth in human neuroblastoma SH-SY5Y cells. In addition, it

enhances insulin-mediated cell growth and protects against high glucose, but not hyperosmolar-induced apoptosis. In the present of 4 nM insulin, C-peptide (3 nM) significantly increases autophosphorylation of the insulin receptor (IR) but not that of the insulin-like growth factor 1 reportor (IGF-IR). It stimulates phosphoinositide 3-kinase (PI-3 kinase) and p38 mitogen-activated protein (MAP) kinase activation, enhances the expression and translocation of nuclear factor-kB (NF-kB), promotes the expression of Bcl2 , and reduces c-jun N-terminal kinase (JNK) phosphorylation in access of that of insulin alone. These observations suggest that C.peptide in the presence of insulin exerts synergistic effects on cell proliferation, neurite outgrowth and has an anti-apoptotic effect. These effects are likely to be mediated via interaction with insulin receptor signaling pathways.

### 094P. The Molecular Abnornalitiers of the Paranode in Type 1 Diabetic Polyneuropathy (DPN) are Prevented by C-Peptide

Sima AAF; Pierson CR; Zhang W

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Axoglial dysjunction is a characteristic structural charge in type 1 human and experimental DPN and accounts for the more severe functional deficits in type 1 DPN. The disruption of the paranodal barrier allows for lateralization of nodal Na-channel resulting in conduction block. The high affinity insulin receptor colocalizes with paranodal axoglial junctions which consist of caspr and cytoskeletal proteins like actin and contactin and are joined by cell adhesive β1 Na-channel subunit which in turn interacts with RTPTβ.

RTPTβ is dependent on insulin and NGF signaling. The SH3 domains of caspr mediate protein-protein interaction by binding to p85 of PI-3 kinase a key intermediary of these signalling pathways.

To examine the possible molecular abnormalities underlying axoglial dysjunction in type 1 DPN, we examined 8 mo type 1 BB/Wor-and type 2 BB/Z-rats and type 1 rats replenished with proinsulin C-peptide. In type 1 rats the expression of caspr, contactin and β1 Na-channel were significantly downregulated, whereas actin and the β2 Na-channel were not. These abnormalities did not occur in hyperinsulinemic and isohyperglycemic type 2 BB/Z-rats and were prevented by insulinomimetic C-peptide in type 1 rats, in whom the paranodal barrier remain intact. From these data we conclude that impaired insulin action in type 1 DPN not only downregulates the expression of several key paranodal moleculares, but also interferes with their assembly and that these abnormalities are preventable by replenishing C-peptide levels in type 1 diabetes.

### 095P. Impaired Endoneural Blood Flow but not Oxidative Stress is Prevented by C-Peptide in Type 1BB/Wor-Rats

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We previously reported that C-peptide prevents and reverses diabetic neuropathy in type 1 BB/Wor-rats. Here we examined the effect of C-peptide on endoneural blood flow and oxidative stress in 2-mo type 1 BB/Wor-rats replaced with II-rat C-peptide from onset of diabetes. The data were compared with non C-peptide replaced BB/Wor-rats, duration matched isohyperglycemic type 2 BB/Wor rat, and non-diabetic control rats. Blood glucose levels were not altered by C-peptide replacement. C-peptide significantly prevented MNCV ($p<0.01$), SNCV ($p<0.001$) and thermal hyperalgesia ($p<0.01$) in BB/Wor-rats. It fully prevented ($p<0.01$) the impaired endoneural blood flow and vascular conductance in type 1 rats. These effects were eliminated by the simultaneous treatment with L-NAME, which were associated with a partial elimination of the effects on nerve conduction velocities. C-peptide had no effects on oxidative stress (MDA), nor did it effect the impaired levels of catalase or SOD in type 1 BB/Wor-rats.

Taken together with previous studies in which C-peptide had no effect on the polyol-pathway, the present data suggest that C-peptide exerts its effects on endothelial NO independently of the polyol-pathway. On the other hand the lack of an effect by C-peptide an oxidative stress parameters suggests that hyperglycemia-induced oxidative stress plays a lesser role in the early pathogenesis of type 1 diabetic neuropathy.

### 096P. C-Peptide Corrects Deficits in Nerve Regeneration in the Type 1 Diabetic BB/W-Rat

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Nerve fiber regeneration is severely effected in insulin and C-peptide deficient type1 BB/W-rats, but not in normo-insulinemic and C-peptidemic type 2 BB/Z-rats. To study the role insulinomimetic proinsulin C-peptide may play in nerve regeneration, type 1 BB/W-rats were supplemented with rat C-peptide from onset of diabetes and compared with non C-peptide-replaced isohyperglycemic diabetic and non-diabetic BB/W-rats. Following sciatic nerve crush injury, early gene responses such as insulin-like growth factor, c-fos and nerve growth factor were examined longitudinally in sciatic nerve. Neurotrophic factors, their receptors and β–tubulin and neurofilament expression were examined in dorsal root ganglia. C-peptide replacement significantly normalized the early gene responses in injured sciatic nerve and partially corrected the expression of endogeneous neurotrophic factors, their receptors as well as neuroskeletal protein expression in dorsal root ganglia. These effects translated into normalization of axonal radial growth and significantly improved axonal elongation of regenerating fibers in C-peptide-replaced hyperglycaemic BB/W-rats. New conclude that impaired insulin action is probably more important than hyperglycemia per se as a mechanism underlying impaired nerve fiber regeneration in type 1v diabetic neuropathy.

### 097P. Copper Sulfate can Stimulate Schwann Cell Proliferation in Sciatic Nerve (a Pilot Study)

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*Background*. The role of vascular endothelial growth factor (VEGF) in axonal regeneration was previously thought to be due to its angiogenic effect but according to recent studies it has come out that this magic molecule also plays direct roles in this process like stimulating proliferation of schwann cells. It has been demonstrated that VEGF prolongs survival and stimulates proliferation of schwann cells. On the other hand, Copper ion has been proved to be the co-factor of VEGF and probably induces synthesis of this molecule. Our theory was founded on these facts: injection of copper

solution increases VEGF production by schwann cells and consequently stimulates their proliferation.

*Method*. Our study involved five groups with five rats in each one. We crushed all rats' left sciatic nerve proximal to its bifurcation and then injected copper sulfate in sciatic nerve of experimental groups (Concentration = 0.5, 1.0, 2.0, 4.0 ngr/ml) and the same volume of stilled water in control group. Ten days after injection all rats were sacrificed and the stained slides of their nerves were observed under light microscope.

*Results*. Both groups showed Wallerian-degeneration but schwann cell proliferation was stimulated in case group (C = 4 ngr/ml) more than in control group.

*Conclusion*. Surprisingly, in spite of previous concept of neurotoxic role of copper, it is deduced by this study that copper has a biphasic effect on peripheral nerves. We suggest that low concentration of copper sulfate can increase VEGF expression by schwann cells and subsequently stimulates schwann cells proliferation which can be used to promote axonal regeneration in peripheral nerve injuries.

### 098P. Comparison of the Immunophenotype of Benign and Malignant Tumors of the Peripheral Nervous System

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*Aim*. This study aims to investigate the immunophenotype of benign and malignant tumors of the peripheral nervous system and the diagnostic and predictive value of the most commonly used immunohistochemical markers.

*Materials and methods*. A retrospective study of 98 Schwannomas and 10 neurofibrosarcomas (1993-2002) was carried out, regarding 41 males and 67 females, aged between 18 and 80 years. The immunohistochemical study included vimentin, smooth muscle actin, HHF-35, desmin, S-100 protein, neuron specific enolase, glial fibrilary acidic protein, synaptophysin, chromogranin, Leu-7, α1-antichymotrypsin, CD-34, CD-68 (clones KP1 and PGM1), epithelial membrane antigen, CAM5.2, proliferating cell nuclear antigen, Ki-67 mitotic index and the p53 accumulation oncoprotein.

*Results*. The most commonly detected markers were vimentin, HHF-35, S-100 protein, neuron specific enolase, α1-antichymothrypsin, CD-34, proliferating cell nuclear antigen, Ki-67 proliferation index,while the rest were positive in a small number of cases. The epithelial markers were useful in the differential diagnosis mainly from the synovial sarcoma as well as clarifying the heterologous epithelial components. CD34positive expression was helpful in identifying the plexiform benign tumors, while the oncoprotein p53, was detected mainly in the malignant tumors and was negative in the benign.

*Conclusion*. The oncoprotein p53 seems to be important in distinguishing the malignant tumors from the benign tumors of the peripheral nervous system and its expression seems to be related to the malignant change and the recurrence of these tumors.

### 098P bis. Effects of a Cell Phone 860 MHz Pulsed Radiofrequency on the Promotion of ENU-Induced Nerve Tumors in Rats

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*Background*. A few reports have implicated mobile phone radio frequencies in the development of brain tumors and cranial nerve tumors. The purpose of this study was to determine if an 860 MHz pulsed radiofrequency (PRF) promoted ethylnitrosourea (ENU) induced brain, cranial or spinal nerve tumors in rats. The negative outcome for brain tumors has been described.

*Materials and methods*. A total of 1080 Sprague-Dawley rats born of mothers given 6.3 or 10.0 mg/kg ENU intravenously on day 15 of gestation were randomized by ENU dose into 3 groups of 360 rats of equal sex. One group was exposed to the PRF, one was sham exposed and one was a cage control. The PRF was delivered 6 hours per day, 5 days per week beginning at 52 days of age. The specific absorption rate was 1 ± 0.2 W/kg average to the brain and cranial nerves. An equal number of rats from each group were randomly selected for euthanasia and necropsy every 30 days between 172 to 322 days of age to determine the effect of the PRF on tumor latency.

*Results and conclusions*. A total of 227 cranial and 189 spinal nerve schwannomas were observed. There was no statistically significant effect of the PRF on tumor latency, malignancy, multiplicity or volume.

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## Brain Tumors: New Clinicopathologic Entities

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The 2000 WHO tumor classification refined nomenclature and grading for existing entities such meningioma; included lesions that were recognized but were not included in the prior classification (atypical teratoid/rhabdoid tumor, tanycytic ependymoma, large cell medulloblastoma, rhabdoid meningioma, and teratoma with malignant transformation); and added entities that had been recently described (chordoid glioma and cerebellar liponeurocytoma). Polar spongioblastoma was downgraded from an entity to a tissue pattern. Among the changes with the most impact on pathologists were those related to grading of meningiomas. Criteria for grades I, II, and III were established, as based on clinicopathologic studies that defined the prognostic significance of histopathological variables. In effect, they tightened criteria for grade III (malignant) meningioma and loosened those for grade II ("atypical") lesions. Chordoid glioma of the third ventricle was placed in the group of glial tumors of uncertain origin since there was insufficient evidence to place it in the astrocytoma category. There was considerable discussion about of well differentiated neuronal and glioneuronal tumors, such as the cerebellar neurolipocytoma and distinction from medulloblastoma. Other recently described lesions such as the papillary glioneurocytoma were considered neurocytic tumor variants, with general agreement that descriptions of new glioneuronal entities will continue to appear. Dysembryoplastic neuroepithelial tumor, including the concept of a "non-specific form," was discussed in detail.

## Lyme Disease

Coyle PK, MD

Lyme disease is a bacterial infection due to a tick-borne spirochete called *Borrelia burgdorferi*. The complete genome of this organism has been sequenced and involves 1.5 megabases, with a single 950-kilobase linear chromosome and a number of linear and circular plasmids. The only vectors involved in documented transmissions are Ixodid (hard body) ticks, so that Lyme disease is geographically restricted. Worldwide, infection has occurred in more than 50 countries. Each year up to 20 000 cases are reported in the United States and 60 000 cases in Europe. Although this is a systemic infection, the targeted body organs are the skin, musculoskeletal, cardiac, and nervous systems. There are suggestive clinical syndromes for each of these target organs. Similar to other human spirochetal disorders (syphilis, leptospirosis, relapsing fever), Lyme disease occurs in stages separated by quiescent periods.

Recent studies have focused on critical organism-vector-host interactions, virulence factors, and pathogenetic host immune mechanisms. There are several instructive animal models of Lyme disease which show distinctive features. None completely mimic human infection, although the rhesus primate model comes closest to recapitulating neurologic disease. This paper will highlight recent advances in these areas, including the issue of tick copathogens.

Neurologic involvement in Lyme disease can affect any part of the neuraxis. There are a limited number of suggestive syndromes associated with early or late stage infection. Early dissemination is associated with meningitis/meningoencephalitis, facial nerve palsy, or acute painful radiculoneuritis. Late stage infection is associated with mild encephalopathy, chronic radiculoneuropathy, or chronic encephalomyelitis. In addition, there are a number of unusual neurologic syndromes reported. Neuropathologic changes associated with Lyme disease involving the central nervous system are not striking. There are extracellular spirochetes, microglial nodules, meningeal and perivascular mononuclear cell inflammation, and mild spongiform changes. Unusual examples of obliterative vasculopathy , demyelination, and granulomatous changes have also been reported. Peripheral nervous system changes include axon damage, epineural, perineural and perivascular inflammation, vasa nervorum angiopathy, and within the muscle focal myositis, interstitial inflammation, focal necrosis, and rare spirochetes.

The lack of a readily available direct infection assay has led to diagnostic and therapeutic issues. Current clinical issues in Lyme disease focus on improved diagnostic testing, preventive strategies including development of new vaccines, and improved understanding and management of the post Lyme syndrome. Recent studies suggest this syndrome is not antibiotic responsive.

This paper will highlight recent basic and clinical advances in understanding Lyme disease. Infection with *B.burgdorferi* serves as an excellent model to study direct and indirect effects of a pathogen on the nervous system.

## Pathophysiology and Treatment of Repeated Thromboembolic Cerebral Insults

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Patients who have suffered recent transient cerebral ischemic episodes are at risk for stroke and can show re-emergence of prior deficits when presented with a pharmacological challenge. Experimentally, the post-thrombotic brain also shows an enhanced vulnerability to mild secondary insults. We tested the hypothesis that deficits in endothelial nitric oxide synthase (eNOS)-dependent dilation and increased matrix metalloprotease (MMP) expression after thromboembolic processes participate in this increased vulnerability and hemorrhagic transformation. Male Wistar rats underwent common carotid artery thrombosis (CCAT) via the photochemical method. At 6, 24, and 72 hours after CCAT, a cranial window was fashioned over the ipsilateral MCA territory to measure eNOS-dependent dilation by applying acetylcholine. eNOS mRNA and protein were measured in segments of the embolized MCA. At 24 hours after CCAT, brain samples were prepared for MMP analysis using immunohistochemical approaches and zymography. eNOS-dependent dilation was significantly reduced at 6 hours, elevated at 24 hours, and returned to baseline at 72 hours after CCAT. eNOS mRNA increased at 2 hours and was followed by a rise in protein at 24 hours. MMP-2 and MMP-9 activity increased at 24 hours after CCAT. Immunocytochemical analysis detected MMP-2 and MMP-9 associated with blood vessels and other cell types in the thrombosed hemisphere. Abnormalities in eNOS-dependent vasodilation and increased MMP expression occur following thromboembolic stroke. Altered vasodilation after CCAT may contribute to increased infarct volumes observed with secondary insults, while elevated MMP expression could underlie the hemorrhagic transformation seen with repeated insults. In clinical stroke,

therapeutic strategies targeting these pathomechanisms may prevent stroke in patients at risk.

### Molecular Pathology of the Motor Endplate

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The motor endplate (EP) has served as a prototype for understanding mechanisms underlying synaptic transmission over the past 50 years. Analysis of the EP in autoimmune myasthenia gravis revealed the acetylcholine receptor (AChR) as the target of pathogenic antibodies. In the Lambert-Eaton syndrome, freeze-fracture EM pinpointed deficiency of voltage-gated calcium channels as the cause of reduced quantal release. In the congenital myasthenic syndromes (CMS) EP studies revealed a diverse array of molecular targets and highlighted their contribution to synaptic function. Clinical observations combined with electrophysiologic and morphologic studies paved the way for detection of mutations in synaptic proteins, and studies of expressed mutants allowed correlation of functional consequences of the mutations with predicted alterations in protein structure. This approach led to the discovery of CMS caused by mutations in choline acetyltransferase (ChAT) and in the collagenic tail acetylcholinesterase (AChE). Kinetic defects in the acetylcholine receptor (AChR), heralded by abnormally prolonged or brief synaptic and single channel currents, resulted in discovery of the slow and fast channel CMS. Another class of CMS with EP AChR deficiency was traced to low-expressor mutations in AChR, or to mutations in rapsyn that plays a key role in concentrating the receptor in the postsynaptic membrane. Finally, a unique mutation in the adult muscle sodium channel (Nav 1.4) that markedly enhances fast inactivation was found to cause a myasthenic phenotype.

### The Value of Transgenic and Gene Targeted Models for Experimental Therapeutics of Neurodegenerative Diseases

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The neurodegenerative illnesses, including Alzheimer's disease (AD), and Parkinson's disease (PD), are associated with characteristic clinical signs, genetic risk factors, dysfunction/death of specific subsets of neurons, and disease-defining pathological/biochemical abnormalities. Transgenic and knockout (KO) studies have been very valuable in understanding disease mechanisms, in identifying potential therapeutic targets, and in designing/testing novel treatments. In autosomal dominant familial AD, mutant genes encoding the amyloid precursor protein (APP) or presenilin 1 (PS1) influence the levels or length of Aβ, 4 kD toxic peptides, which are generated by cleavages of APP by β-secretase 1 (BACE 1) and by β-secretase activities. Aβ accumulates in the brain and Aβ deposits are at the extracellular core of neuritic plaques in the brains of individuals with AD. Mice overexpressing mutant *APP*/*PS1* develop age-associated increases in levels of brain Aβ, neuritic plaques within the hippocampus and cortex, and behavioral deficits, particularly in working memory. To illustrate how knockout strategies are used to identify therapeutic targets, we describe work in which we targeted genes encoding proteins critical for pro-amyloidogenic secretase activities. Studies of *BACE1* -/- mice demonstrate that BACE1 is the neuronal β-secretase; without BACE1, Aβ is not produced. Moreover, *APPswe*; *PS1δE9*; *BACE1* -/- mice do not form Aβ in brain, nor do they show memory deficits. Parallel KO studies have disclosed that PS1 and NCT are key components of β-secretase activities and theses proteins may also be targets for inhibition of formation of Aβ. In PD, mutations in α-synuclein (α-syn) are implicated in a subset of familial cases, and α-syn is also a principle component of Lewy bodies/neurites. Enzymatic cleavages of α-syn appear to generate intracellular truncated toxic peptides similar to the Aβ-generating secretases. A53Tα-Syn mice develop motoric deficits, ubiquitinated α-syn inclusions in neurons, α-syn peptide fragments and peptide aggregates. The hypothetical "synucleinases" responsible for these cleavages may represent targets for treatment of PD. These results of studies of these transgenic and KO models have significant implications for the development of mechanism-based therapies designed to prevent accumulation of Aβ in individuals with AD, and possibly other toxic peptides of neurodegenerative diseases like PD.

### Neurodegenerative Diseases Caused by the Deposition of Proteins with an Abnormal Carboxy Terminus

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A number of neurodegenerative diseases have been discovered in which the genetic defect consists of a point mutation in the codon that is normally the stop codon or a duplication-insertion in the coding region of the gene. These changes result in the production of proteins that contain a carboxy terminus that is abnormal for both length and primary sequence. These genetic abnormalities lead to the intracellular and/or extracellular accumulation of abnormal proteins. We will illustrate the molecular genetic, biochemical and neuropathologic aspects of these phenomena. We will focus on two groups of diseases. The first group includes the so-called "familial British and Danish dementias." In this group, the molecular genetic defects reside in the BRI gene in chromosome 13. The mutant proteins and their degradation products accumulate in the extracellular space leading to the formation of plaques and amyloid vascular deposits. The second group includes the so-called "ferritinopathy." In this group, the molecular genetic defects reside in the ferritin light chain gene in chromosome 19. The mutant proteins and the wild-type light and heavy chain ferritin are present in the intracellular inclusions. These studies have contributed important information to understanding different molecular mechanisms of neurodegeneration leading to dementia and allowed us to classify neurodegenerative diseases according to their molecular characteristics.

## Current Issues in Multiple Sclerosis and the Role of Pathology

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Pathology has played a central role in understanding the recent explosion of advances in multiple sclerosis at basic, clinical, diagnostic and therapeutic levels. Dissection of the elements of the pathology of the lesion and the surrounding brain is central to a rational approach to understanding the inflammatory and immune factors which cause the disease, lead to clinical symptomatology and the design of imaging diagnostic techniques, as well as to understand the effects on resolution, healing, including remyelination and gliosis, and therapy. The inflammatory process is considered to be one of the earliest initiators of the lesion, and has both specific and generalized targets in the CNS. Whereas both myelin and the axon may be damaged in this process, it is not clear whether the latter represents a primary or secondary target. Current imaging techniques aim at detecting this both in and outside the lesion. The role of inflammation in the ongoing destruction of axons that is considered to be a major cause of progression is unclear, and remains an important issue for therapeutic intervention. Similarly although imaging modalities have highlighted the changes occurring outside the plaque, and their possible importance in pathogenesis as well as clinically, the true nature of these changes requires careful, temporal, comparative pathological examination. These and other issues will be the focus of the talks in this symposium.

## Immune Trafficking in the Brain

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We have traced the migratory pathways of encephalitogenic T-cells before, during and after clinical Experimental Autoimmune Encephalomyelitis in the Lewis rat. Our experimental paradigm relies on the study of retrovirally transduced GFP expressing ("green") myelin autoaggressive CD4 T-cells combining serial 2-photon imaging with real-time assays of gene transcription and protein expression. Our data indicate that encephalitogenic T-cells, that must be maximally activated in vitro for transfer of EAE, radically re-program their gene expression pattern during a first 72 hrs. prodromal period within peripheral immune organs, by down-regulating activation markers and up-regulating chemokine receptors ("migratory phenotype"). About 72 to 96 hours post transfer, large numbers of migratory T-cells invade the CNS via the blood-brain-barrier. One major part of the cells rapidly zigzags through the brain parenchyma, while the other cells seem to be attached to local structures. There is evidence that the attached cells recognize locally presented autoantigen. These effector cells down-modulate their T-cell receptor complex and re-express activation markers, along with pro-inflammatory cytokines and chemokines ("effector phenotype"). Intra-parenchymal T-cell activation seems to underlie the pathogenic inflammatory response responsible for clinical EAE. Shortly after re-activation, most, if not all of the green T-cells undergo apoptotic cell death. Their depletion from the CNS coincides with termination of the clinical EAE episode.

## Axonal Pathology in Multiple Sclerosis

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There is increasing evidence from morphological studies that axonal pathology such as axonal transections or acute axonal damage occurs in multiple sclerosis (MS) lesions. This is supported by recent magnetic resonance imaging (MRI) and magnetic resonance spectroscopic (MRS) studies which described hypointense lesions in T1-weighted MRI scans or decreases in the neuroaxonal metabolite N-acetylaspartate (NAA) in MS suggesting that axonal damage is responsible for the persistent neurological deficit in MS patients. The present talk will therefore cover the following topics: How extensive is axonal reduction in MS lesions? Is there a MRI or MRS correlate for axonal loss? How can acute axonal damage be quantified and which immunopathological mechanisms are involved?

Axonal reduction in MS occurs early in plaque development as well as early in the course of the disease; however, there are major variations in axonal reduction in individual MS patients. Axonal loss correlates with the degree of hypointensity in T1-weighted MRI, hypointensity is additionally affected by extracellular edema and the rate of demyelination or remyelination. Decreased NAA levels in MRS show a clear correlation with axonal reduction in MS lesions. Acute axonal damage can reliably be quantified by immunocytochemical detection of the Alzheimer precursor protein (APP).

APP-positive structures were detected in all stages of demyelinating activity indicating that acute axonal damage probably occurs independent of active demyelination. The relevance of axonal loss for the development of clinical symptoms is investigated in cases of clinically silent MS. Different factors such as lesions site, axonal preservation or remyelination contribute to the clinical non-appearance of MS lesions.

## The Role of MR Imaging in Defining MS Pathology In Vivo

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Conventional magnetic resonance imaging (cMRI) is widely used for diagnosing multiple sclerosis (MS) and monitoring disease activity and evolution in natural history studies and clinical trials. However, the correlation between cMRI and clinical findings is far from being strict and such a discrepancy is even more evident when moving from the setting of large-scale studies to the management of individual patients. Among the reasons for this "clinical-MRI paradox," a major role has been attributed to the limited specificity of cMRI to the heterogeneous pathological substrates of MS and to its inability to quantify the extent of damage in the normal-appearing tissues.

Modern quantitative MR techniques have the potential to overcome some of the limitations of cMRI. Metrics derived from magnetization transfer and diffusion-weighted MRI enable us to quantify the extent of structural changes occurring within and outside macroscopic MS lesions with increased pathological specificity over cMRI. MR spectroscopy can add information on the biochemical nature of such changes, with the potential to improve significantly our ability to monitor inflammatory demyelination and

axonal injury. Finally, functional MRI might provide new insights into the role of cortical adaptive changes in limiting the clinical consequences of white matter structural damage.

Although the application of modern MR techniques is changing dramatically our understanding of how MS causes irreversible disability, their use for clinical trial monitoring and clinical practice is still very limited. Whereas there is increasing perception that modern quantitative MR techniques should be more extensively employed in clinical trials to advance the understanding of MS, their use in clinical practice should still be regarded as premature.

## PLATFORM PRESENTATIONS

### 099. Axon Loss in Multiple Sclerosis: A Pathological Survey of the Corticospinal and Sensory Tracts

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*Background*. Pathological studies in multiple sclerosis (MS) have classically emphasised the relative preservation of axons in comparison to myelin. Recent evidence, however, demonstrates that axon loss is also significant, affects long tracts such as the corticospinal and sensory tracts and relates closely to functional disability. Accordingly, the cause of this axonal loss is the focus of the current investigation.

*Materials and methods*. Post-mortem material of 55 MS patients and 32 matched controls was used to examine quantitatively the population of axons in the corticospinal tracts from the medulla to the lumbar spinal cord and the sensory tracts from the lumbar to upper cervical spinal cord. Myelin and axon-stained sections have been prepared to estimate the notional area and axon density respectively of both tracts.

*Results*. Our results indicate that in the corticospinal tracts there is a significant reduction of the area and axon density at all levels investigated in MS cases when compared to controls. In contrast, the sensory tracts in MS cases showed a significant reduction in area and axon density only in the upper regions of the spinal cord. Of the fibers lost in MS, we have found that small fibers (<3 μm) seem to be particularly affected with large fibres remaining relatively preserved.

*Conclusions*. In MS, axon loss is significant and occurs throughout the length of the neuraxis.

### 100. Patterns of Grey Matter Demyelination in Multiple Sclerosis (MS)

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Though involvement of the grey matter in MS is well-documented, it never attracted much attention. We report 3 types of demyelination in the grey matter and characterized the cell content of the tissue, of the overlying meninges and the type of lesions in the white matter of the patients studied. The tissue was studied using histochemical and immunohistochemical techniques, identifying cell types and myelin content.

The 3 patterns we found were: I) lesions involving both white and grey matter; II) circumscribed lesions entirely located within the grey matter; and III) an extensive, ribbon-like demyelination of the cortical surface, involving layers 1 to 4 or 5. The type I lesions were similar to the white matter lesions, containing (dependent on stage) inflammatory cells, mainly macrophages. The type II lesions were difficult to recognize without a myelin stain. They show a (very) mild hypercellularity, but with few macrophages. The type III lesions can only be identified in myelin stains. Meningeal cellularity is increased in MS patients, irrespective of the underlying lesions, though the extent of cell increase appears to be higher in meninges directly overlying a lesion.

The clinical importance of cortical demyelination remains to be established.

### 101. The 14-3-3 Protein is Expressed in Reactive Astrocytes in Demyelinating Lesions of Multiple Sclerosis

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*Objective/Background*. The 14-3-3 protein is a family of evolutionarily conserved, acidic 30-kDa proteins enriched in the CNS with the localization primarily in neurons. Seven isoforms of this family form a dimeric complex which acts as a novel type of molecular adaptor capable of interacting with key components in the Ras/Raf/MAPK signaling pathway. Detection of the 14-3-3 protein in the CSF provides a reliable biochemical marker for the premortem diagnosis of Creutzfeldt-Jacob disease, although it is occasionally deteced in the CSF of prion-unrelated neurological diseases such as meningoencephalitis, and it is expressed not only in neurons but also in glial cells in mouse brain cell cultures (Satoh J et al, *Eur Neurol* 41:216-225). However, at present, it remains unknown whether the 14-3-3 protein plays a role in the pathogenesis of multiple sclerosis (MS).

*Methods*. To investigate the expression pattern of the 14-3-3 protein in MS lesions, brain tissues derived from autopsy of 4 cases of chronic progressive MS were stained with a panel of anti-14-3-3 protein isoform-specific antibodies (Santa Cruz Biotechnology).

*Results*. The cell bodies and processes of neurons in various regions were labeled at variable intensities with K-19 antibody which reacts with all isoforms, while the CNS myelins were usually unstained. Reactive astrocytes accumulated in chronic active lesions were intensely stained with C-16, C-17, and T-16 antibodies specific for zeta, theta, or epsion isoforms, respectively. Macrophages infiltrating in chronic active lesions were also labeled with C-16 anti-zeta isoform antibody. By Western blot analysis, the levels of expression of the 14-3-3 protein were elevated markedly in proliferating newborn mouse astrocytes incubated in the 10% FBS-containing culture medium compared with the growth-arrested astrocytes cultured under the serum-free conditons. Conclusions: These results suggest that the 14-3-3 protein expressed in reactive astrocytes might regulate proliferation of astrocytes which promotes glial scar formation in MS lesions.

### 102. Multiple Sclerosis: Immunoregulation of Oligodendrocytes by Microglia

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The molecular pathway(s) underlying oligodendrocyte depletion in multiple sclerosis (MS) remain unknown. Previous studies have demonstrated a wide array of pro-inflammatory, developmental and cell-fate receptors on oligodendrocytes in MS, but none with specificity for MS. We have examined by immunocytochemistry and Western blotting, pro-inflammatory (Th1-type) and immunoregulatory(Th2-type) cytokines and their receptors in CNS tissue from

MS, non-MS and normal subjects to determine whether oligodendrocytes display aberrant expression which may account for their demise in MS. Results have shown in all types of CNS tissue studied, that oligodendrocytes display an unusual affinity for both Th1- and Th2-type cytokine receptors (IFNgR/IL-12R/IL-18R and IL-4R/IL-6R/IL-10R, respectively). Microglia in the same tissue stained positively for the same receptors and the corresponding cytokines, suggesting both autocrine and paracrine mechanisms. Increased levels of cytokine receptors (both Th1- and Th2-type), occurred around MS lesions and this almost invariably correlated with similar levels of expression of the corresponding cytokines on microglia. Except for IL-18, oligodendrocytes were never seen to express cytokines. Some Th2-type receptors were also demonstrated on oligodendrocytes cultured from human fetal spinal cord and ongoing experiments are testing their functionality. The affinity for oligodendrocytes and microglia to express cytokine receptors suggest the existence of molecular pathways between these 2 cell types of fundamental relevance to pathogenesis.

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## POSTERS

### 103P. Identification of Relapse-Relating Factors in Chromic Relapsing Autoimmune Encephalomyelitis

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Multiple sclerosis is characterized by the presence of relapse of clinical signs and demyelinating lesions in the central nervous system (CNS). Using its animal model, chromic relapsing experimental autoimmune encephalomyelitis (CR-EAE), we tried to identify cytokines and chemokines that are involved in the relapse of the disease. Acute and CR-EAE was induced in DA rats by immunization with purified bovine myelin at low (1 mg) and high (5 mg) doses, respectively. At different time points, spinal cords were removed and the levels of cytokine and chemokine mRNA were determined by competitive PCR. We examined IFN-β, TNF-α, IL-12, IL-4, IL-10, MCP-1, MIP-1α, RANTES and IP-10 mRNA and found that only IFN-β and IP-10 were significantly different between the acute and CR-EAE groups.

Namely, IFN-β during the first attack and IP-10 at the second attack of CR-EAE were significantly higher those that at the peak stage of acute EAE. These findings suggest that IP-10, which is a lymphocyte and macrophage chemoattractant factor, and its upregulator, IFN-β, play an important role in the relapse of EAE. In accord with this finding, pathological examination revealed that far more macrophages infiltrated in the CNS of rats with CR-EAE than with acute EAE. Based on these findings, we are currently investigating the effects of DNA vaccine encoding CXCR3, a main receptor of IP-10 whether it suppresses or ameliorates the relapse of EAE.

### 104P. CX3Cl1 and CX3CR1 Expression in Human Brain Tissue of Multiple Sclerosis and Control Patients

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We compared the immunohisto/-cytochemical expression of CX3CL1 and its receptor in human brain tissue derived from control and multiple sclerosdis (MS) patients, and on cultures of glial cells. In addition a chemotaxis assay was performed with microglial cells and CX3CL1 to assess the functionality of the chemokine.

Results show a constitutive expression of CX3CL1 and CX3CR1 by astrocytes both in vivo and in vitro. Microglial cells only expressed CX3CR1, and they responded to CX3CL1 in the chemotaxis assay. Expression of CX3CL1 is upregulated in response to proinflammotory cytokines, but no expression difference was found between MS and control patients.

The data seem to suggest a more general physiological role for CX3CL1 in the central nervous tissue, occurring also in absence of proinflammatory conditions.

### 105P. Neutralizing Antibodies Antagonise the MMPs Suppressive Effect of IFNβ

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Some Multiple Sclerosis (MS) patients develop Neutralizing Antibodies (NAb) during Interferon-beta (IFNβ) treatment. Here we evaluated the impact of NAb against IFNβ, measuring mRNA of matrix-metalloproteinase-2 (MMP-2), MMP-9 and MxA by real-time PCR in peripheral blood mononuclear cells from 39 treatment-naive MS patients, and 68 on-treatment patients. IFNβ-induced NAb were evaluated by CPE assay, quaterly. Three groups of patients were identified: NAb-negative (NAb-), persistent NAb-positive (pNAb+; ≥2 consecutive samples positive), and isolated NAb+ (iNAb+).

MMPs were expressed in most of the treatment-naive patients and after two IFNβ injections levels remained unchanged. In contrast, mean MxA levels increased 10-fold after IFNβ injection, as compared to baseline levels ($p<0.0001$).

During treatment Nab- and iNAb+ patients showed MMPs expression lower than in treatment-naive patients ($p<0.0001$), and in pNAb+ patients ($p<0.05$).

Induction of MxA remained at baseline levels in 18/68 treated-patients: 13 of these were pNAb+. In pNAb+ patients MxA expression was lower compared to NAb- ($p<0.0001$) and iNAb+ ($p=0.0441$) patients and reached levels similar to those in untreated subjects ($p=0.1422$).

Conclusions: *i)* Suppression of MMP expression is a long-term, but not an acute effect of IFNβ therapy, *ii)* NAb antagonise the MMP suppressive effect of IFNβ, and *iii)* MMP-9 is the first marker of IFNβ biological action that has a role in MS pathogenesis.

### 106P. α-Lipoic Acid is Effective in Prevention and Treatment of Experimental Autoimmune Encephalomyelitis

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α-Lipoic Acid (αLA) is a neuroprotective metabolic antioxidant which crosses the blood brain barrier and has inhibitory effects on matrix-metalloproteases (MMP). We tested the effects of αLA for its ability to prevent MOG induced EAE. Daily oral administration of αLA, starting at the time of immunization, significantly prevents EAE progression compared to control mice. This was associated with a reduction both in the brain and spinal cord of perivenular inflammatory foci containing macrophages and T-cells, as well as reduced demyelination.,We then tested its ability to treat EAE after onset of disease. Oral administration of αLA following disease onset did not ameliorate EAE. In contrast, daily intraperitoneal (IP) administration of αLA at a higher dosage strongly and significantly prevented EAE disease progression when compared to controls, with a significant reduction of demyelination and inflammatory infiltrates. We examined also the cytokine production by MOG specific T-cells, observing a decreased production of both Th1 and Th2 cytokines. Our data indicate that αLA can effectively interfere with the autoimmune reaction associated with EAE and supports further studies as potential therapy for MS.

### 107P. Erdheim-Chester Disease (ECD) Simulating Multiple Sclerosis (MS)

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*Introduction*. ECD is a rare form of non-Langerhans histiocytosis of unknown etiology. It typically involves long bones and to a variable extent other organs occasionally including CNS. Reports on morphologically verified brain lesions in ECD are rare and have been based on autopsy studies. The initial clinical presentation of ECD with neurological symptoms is exceedingly rare. We now describe the clinical, radiological and pathological features of such a case in which the diagnosis was made by biopsy.

*Clinical and pathological findings*. A 27-year-old woman with non-localizing neurologic manifestations was found to have bilateral multiple discrete white matter lesions on CT scan suggestive of MS. A small biopsy sample showed the presence of lipid-laden macrophages and reactive astrocytes interpreted to be consistent with an active demyelinating process. In the ensuing months the patient developed progressively worsening neurologic deficits accompanied by seizures and diabetes insipidus. CT and MRI demonstrated widespread homogeneously enhancing lesions in white matter as well as in gray matter of cerebrum and cerebellum. An adequate biopsy of a lesion showed collections of numerous histiocytes mixed with lymphocytes, plasma cells and a variable number of eosinophils and neutrophils.The histiocytes were strongly positive for CD68 and uniformly negative for S-100 protein and CD1a characteristic of ECD.

*Conclusion*. This case indicates that ECD should be included in the differential diagnosis of multiple enhancing brain lesions.

### 108P. Acute Leucoencephalopathy in Lethal Salicylate Intoxication

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*Background*. Central nervous system dysfunction which may be lethal is common in severe salicylate intoxication, but the pathophysiological mechanisms are largely unknown and, to our knowledge, a detailed histopathological investigation is not available so far.

*Case report*. A 34-year-old mildly oligophrenic woman was admitted to the intensive care unit in comatose state with marked tachypnea. History revealed the oral ingestion of a large amount of acetylsalicylate to attenuate ear pain 12 hours ago. Laboratory investigations showed metabolic acidosis (pH = 7,30; HC03- = 9,4), mildly elevated serum natrium (153 mmol/l) and a leucocytosis of 17 000/microl with left shift. Salicylate in serum measured 668 microg/ml (toxic range). Oxygenation and blood level of lactate and ketonic bodies were normal, as were blood pressure, electrocardiography and CT of thorax and brain. The patient was intubated, fluid and bicarbonate was given intravenously. Six hours after admission asystolia refractory to resuscitation led to death.

*Pathology*. Autopsy showed venous congestion and edema of the brain as well as pulmonary edeme and cardiac dilatation. Histopathology of the brain revealed a diffuse acute leucoencephalopathy with disintegration of myelin sheaths and axonal spheroids in the deep white matter of forebrain and cerebellum. Ischemic neuron changes in cortex and deep nuclei were sparse. Immunohistochemistry showed marked expression of activated caspase 3 in glial cells surrounding the damaged myelin sheaths, indicating apoptotic cell death. In the adjacent cortex apoptosis was absent.

The present case shows, that salicylate intoxication can result in acute leucoencephalopathy. Possible mechanisms of salicylate-induced brain damage are discussed.

## PLATFORM PRESENTATIONS

### 109. The Changes of Characteristics of Afferent Neurocytes Under the Chemical Symphathectomy

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The effect of symphatholytic guanethydine on afferent neurocytes of ganglion nodosum of vagal nerve were investigated. The experiences were conducted on 25 adult femail rats of weight of 200 to 220 g. The intraparietal injections of guanethydine were made daily during 5.5 weeks. The medicine doze was 75 mg/kg of weight animal one. The samples of ganglia were taken at once after ending of rate of guanethydine treatment and on 14th, 30th, 90th, 180th day after that. The metric parameters of form and size of neurocytes were received from half-thin histological layers with the help of the videoanalyzer "Bioscan." In our research the quantytative and qualitative chenges of neurocytes of ganglion nodosum (on relation to control) was established. Qualitative changes are dystrophycal in nature. It is emerged at 14th day after ending of rate of guanethydine treatment and accrue to 180th day. Quantitative metric characteristics are authenticaly changed on relation to control. The middle size of cells was being increased after the guanethydine treatment, had reacheded the maximum to 14th to 30th days and then by degrees was being decreased. The guanethydine treatment causes the destruction of part afferent neurocytes to 90th to180th days. It runs counter to given about strict selectivity of guanethydine treatment effect on adrenoceptive neurocytes. It is apparent that the toxical effect of guanethydine on afferent neurocytes with the use of such symphathectomy technique is observed.

### 110. Neuron Diversity in the Human Medial Mamillary Nucleus

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*Introduction*. The medial mamillary nucleus forms part of the Papez circuit which has been implicated in the functioning of episodic memory. GABAergic interneurons have been described in other Papez circuit structures (cingulate cortex, anterior thalamus) of the human brain. Many of these neurons are co-localised with one of the common calcium-binding proteins. Thus, the immunohistochemical localisation of glutamic acid decarboxylase (GAD) and three calcium-binding proteins was investigated in the medial mamillary nucleus of the human brain.

*Methods*. Using postmortem normal control tissue (n = 8 cases), GAD-immunoreactive (IR) neurons with both simple and multipolar morphologies were found sparsely distributed throughout the MMN.

*Results*. A somal size analysis demonstrated that GAD-IR neurons typically had smaller average somal diameters compared with the total MMN population, suggesting that these neurons represent GABAergic interneurons. A considerable amount of punctate GAD-IR was present within the neuropil of the MMN. Parvalbumin (PV) IR was found in many large diameter neurons with multipolar morphologies. Similarly, calretinin (CR) IR was found in the majority of MMN neurons at a range of intensities. Results of double-labelling experiments revealed no colocalisation between GAD-IR and either PV-IR or CR-IR.

*Discussion*. These results suggest a greater complexity of neuronal subtypes within the human MMN than was previously known.

### 111. Neurones Expressing KN-1 Receptor of the Rat GP

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NK1 is expressed by striatal cholinergic and nitrinergic interneurones and by projection neurones as well. Recent evidences demonstrated the presence of SP-containing fibers directed to the GP. We studied by means immunohistochemistry at the light and confocal microscopy *i)* the expression of NK-1 among pallidal neurones subtypes and *ii)* to study the relationship between neurones expressing NK-1and SP containing fibers. Single label study for NK-1 demonstrated the presence of immunopositive neurones within the GP. Almost all neurones of the GP were immunolabelled for NK-1. GP neurones immunopositive for NK1 were distributed without a particular pattern along the rostro-caudal extent of the nucleus. Dual label study was performed by means of confocal microscopy to study the different distribution of NK1 immunoractivity among the pallidal subtypes identified by their content of the calcium binding protein parvalbumin, which is contained in almost the 60 to 70% of pallidal neurones This study showed that almost all neurones of the GP express the NK-1.The dual label study for SP and NK-1 showed that fibers containing SP innervates a region of the GP located in more caudal and medial region although virtually all neurones express NK-1. These finding indicate that SP acts on virtually all neurones of the GP. The activity is related to the release of the peptide rather than on a direct relationship between neurones and fibers.

### 112. Immunohistochemical Demonstration of Tyroxinhydroxylase Positive Nuclei in the Rat Hypothalamus

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*Background*. Tyroxinhydroxylase (TH) has been documented in various nuclei of the human and animal brain; technical refinements of the method of immunohistochemistry nowadays allows the demonstration of whole maps of positive neuronal assemblies and fiber tracts. In rats, this method has especially been used in the analysis of experimental models of Parkinsons disease.

*Methods*. TH expression was investigated in serial sections of the rat brain (strain BD IX) both by fluorescent and PAP immunohistochemistry. A comprehensive picture of all dopaminergic (and serotonergic) nuclei and pathways emerged. This contribution especially deals with the hypothalamic sites of positive expression, where several patterns of cellular expression can be displayed.

*Results*. Hyxpothalamic nuclei—eg, the nucleus arcuatus and the periventricular nuclei—show a diffuse TH staining within their neuronal perikarya, whereas the nucleus supraopticus in its different compartments as well as the paraventricular nucleus display a

coarse granular staining behaviour. The same type of expression is found in an intermediate zone in scattered neurons invariably assembled around vessels, that are found next to the fornix precommissuralis. The latter can be visualized by additional myelin staining. Vessels and positive cells form a curved line connecting those structures.

*Conclusions*. Morphology and expression pattern point to a former common precursor and function of the supraoptic and paraventricular nuclei. They may have been separated by the formation of the white fiber tract of the fornix. Further, the close connection of the perikarya of both nuclei to small vessels suggest hormon activity into the blood system in addition to their known functions.

### 113. GABAergic Mechamisms in Tractus Solitarius Nucleus on Baroreflex Sensitivity in Acute Hypertension Rat

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Considerable evidence exists that GABAergic mechanisms have important role in control of blood pressure and heart rate, but there is some controversy about which GABA receptor subtype is involved in cardiovascular regulation within the NTS. In this study the baroreceptor reflex was evaluated in anesthetized rats with microinjection of Bicuculine (GABAA antagonist) and phaclofen (GABAB antagonist) into the nucleus tractus solitaius (NTS). Phenylepherine (PE) (1, 2, 4, 8, 16 microg/kg, i. v.) elicited pressor and bradicardic responses. Extracellular recording of baroreceptive neurons in the NTS during application of PE were monitored in anesthetized rats.Regression analysis of the baroreflex curve, revealed a significantly smaller baroeflex sensitivity in Bicuculine receiving group compared with sham. The baroreflex sensitivity was not affected by phaclofen. In extracellular recording the firing frequency of baroreceptive neurons in NTS were increased during microiontophoretic application of Bicuculine but not with phaclofen. Bicuculine exited 71 (83%) of 86 NTS neurons without affecting the remaining 13 neurons (17%). These result demonstrate that GABAA receptors are involve in baroreflex sensitivity and spontaneous discharge in the great majority of the NTS baroreceptive neurons.

### 114. Human Glucose Transporter 5 (hGLUT5) Immunostaining is Useful for Observing Microglial Morphology and for Estimating the Origin of Brain Macrophages

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*Purpose*. In neuropathological studies, it is important to detect both resting and reactive microglia, and also to determine the origin of brain macrophages. We report here that hGLUT5 is sensitive and specific microglial marker in paraffin sections.

*Materials and methods*. We used hGLUT5 rabbit antibody (IBL) against its C-terminal 20 amino acids. Paraffin sections from brains and non-neural tissues, fixed with 4% formaldehyde or 10% formalin, were immunostained for hGLUT5 or other microglial markers after the antigen retrieval.

*Results and discussion*. The hGLUT5 immunostaining demonstrated both resting and reactive microglia with their fine processes, even after one year-long fixation in formalin. As compared with other microglial markers, KP-1, Ki-M1p, CR3.43 and RCA-1, the hGLUT5 antibody could be considered good morphological marker. The hGLUT5 and glial fibrillary acidic protein labelings did not overlap each other in the double immunofluorescent analysis. The hGLUT5 antibody did not labeled most of monocyte-derived brain macrophages in the center of brain infarction, whereas it consistently labeled reactive and phagocytic microglia in the margin of the infarction. Furthermore, most of non-neural histiocytes/macrophages were hGLUT5-negative.

*Conclusion*. The hGLUT5 immunolabeling is highly specific to microglia, and sensitive enough for detecting both resting and reactive microglia in paraffin sections, and thus useful in the routine neuropathological studies.

## PLATFORM PRESENTATIONS

### 115. Sporadic Creutzfeldt Jakob Disease: Molecular, Neuropathological and Clinical Correlation of 17 Cases

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*Background*. It is noteworthy that codon 129 polymorphism Met/Val plays a key role in phenotypic variations of sporadic Creutzfeldt Jacob disease (sCJD). We studied 17 patients who died in southwest of France from 1999 to 2002.

*Methods*. Neuropathological lesions (spongiosis, gliosis, neuronal loss) were semiquantitavely assessed. PRNP genotype, $PrP^{sc}$ presence on Western Blot, and clinical features were determined.

*Results*. Met/Met (11 cases, mild to moderate lesions, diffuse or focal spongiosis, disease duration <6 months, initial frontal symptoms) contrasted with Mel/Val (4 cases, marked lesions in temporal, parietal cortex and parahippocampal gyrus, laminar pattern of spongiosis and disease duration >6 months, aphatia at onset) and with Val/Val (2 cases, moderate lesions in substantia nigra, marked lesions in striatum and thalamus, extra pyramidal symptoms during disease).

Two atypical cases were noted, Met/Val type 1 and Met/Met type 2, unusually associated with kuru plaques in cerebellar and cerebral cortex.

*Conclusion*. Our results are in agreement with a susceptibility of homozygous Met/Met toward sCJD as well as influence of the genotype at codon 129 on neuropathological profile and on clinical signs. They emphasized the interest of such phenotypical and molecular studies to detect new cases and better characterize these diseases.

### 116. Creutzfeldt-Jakob Disease (CJD) Associated with V210I PRNP Mutation: Phenotypic and Molecular Genetic Analysis

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The systematic analysis of the PRNP gene in Italian patients with sporadic CJD revealed V210I mutation in 20 subjects. This mutation was found in other 3 patients with familial CJD. Three cases were homozygotes for the mutation and MM at codon 129 (age at death 45, 50 and 64 years). Twelve of the twenty V210I-heterozygotes were 129MM (mean age 57.5±9.2), seven were 129MV (mean age 58.8±10.9) and one was 129VV (age 75). The neuropathological study of 7 patients (two V210I-homozygotes/129MM, four V210I-heterozygotes/129MV and one V210I-heterozygote/129MM) evidenced variable extend of spongiform changes, neuronal loss, astrogliosis and microglial activation. Western blot analysis of $PrP^{res}$ and PrP-immunohistochemistry showed the presence of type 1 $PrP^{res}$ and a synaptic pattern of PrP-immunoreactivity diffuse to the cerebral cortex, striatum, thalamus and cerebellum in all patients. No consistent differences in age of onset and disease duration were observed between V210I-homo- and heterozygotes. Peculiar clinical features were signs of asymmetrical involvement of the cerebral hemispheres at onset in 3 patients and striking cerebral atrophy in 2 cases with very long disease duration (36 months). This study demonstrates a high frequency of V210I PRNP mutation in sporadic CJD in Italy, providing evidence of phenotypic heterogeneity of CJD associated to this mutation.

### 118. Oligodendrocytes Within Astrocytes ("Emperipolesis") in the White Matter in Creutzfeldt-Jakob Disease

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*Background*. The occurrence of oligodendrocytes within astrocytes ("emperipolesis") has been described in demyelinating lesions in cases of acute multiple sclerosis and other demyelinating disorders. We detected this peculiar finding in the cerebral white matter in Creutzfeldt-Jakob disease (CJD).

*Materials and methods*. Eight consecutive autopsy cases of CJD, which were culled out from the autopsy files of our hospitals, were reviewed histopathologically.

*Results*. All cases exhibited classical histopathological features of CJD. In 5 cases, the white matter was very severely involved, and both axons and myelin sheaths had been lost markedly in most regions of the deep cerebral white matter. Within this devastated white matter, many hypertrophic astrocytes were found to engulf one to several oligodendrocytes within their cytoplasm. This finding was observed also in the cerebral cortex and the cerebellar white matter, albeit to a far lesser degree. In the other 3 cases, the cerebral white matter was relatively well preserved, and "emperipolesis" was not found.

*Consultation*. We found the frequent occurrence of "emperipolesis" (oligodendrocytes within astrocytes) in the white matter of CJD patients. The prevalence of this phenomenon was well correlated with the severity of the white matter degeneration in this disorder.

### 119. Detection of Cellular Prion Protein Gene Variants using Denaturing High-Performance Liquid Chromatography

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Genetic variants of the cellular prion protein gene (PRNP) have been described in familial forms of spongiform encephalopathies. Some polymorphisms in PRNP, such as at codon 129 (M/V) are related with susceptibility to the new variant of Creutzfeldt-Jacob disease and has been implicated with the role of familial prion diseases. Herein, we established the conditions to detect PRNP genetic variants using denaturing high-performance liquid chromatography (DHPLC) which has been successfully used for mutation screening in several disease-related genes.

*Methods*. Unpurified PCR products from human PRNP open reading frame were obtained using strategically positioned primers and

subjected to denaturing and reannealing steps to ensure adequate formation of heteroduplex which permitted the detection of single base substitutions and small deletions. We found specific chromatograms for variant alleles at codons 129 (M/V), 171 (N/S) and octareaped deletion and also for prion disease associated mutations at codons 102 (P/L), 183 (T/A) and 210 (V/I).

*Results*. The data showed concordance with results obtained using DNA direct sequencing and restriction endonuclease digestion. Therefore, DHPLC is a rapid, high sensitive, specific technique and could be used for screening PRNP genetic variants.

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### 120. Prion Protein Deposition in Peripheral Nervous System of Human Prion Diseases

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*Objectives*. To investigate abnormal prion protein (PrP) deposition in peripheral nervous system (PNS) of human prion diseases.

*Methods*. We examined 3 patients with sporadic Creutzfeldt-Jakob disease (sCJD), 2 with dural graft-associated CJD (dCJD), one with Gerstmann-Sträussler-Sheinker disease (GSS) with a PrP P102L mutation (GSS102), and 2 with a P105L mutation (GSS105) by immunohistochemical studies and western blot analyses. An atypical case of sCJD with PrP plaques clinically presented with peripheral neuropathy.

*Results*. In immunohistochemical studies with an anti-PrP monoclonal antibody (3F4), granular PrP deposited in some neurons of dorsal root ganglia (DRG) and a few fibers of peripheral nerves and spinal nerve roots in one sCJD and two dCJD patients, but not in GSS102 or GSS105 patients. The atypical case of sCJD with peripheral neuropathy showed demyelination in 12% of teased fibers, but no obvious PrP deposition in the nerves. Western blot analysis of the PNS from the CJD patients proved a small amount of $PrP^{Sc}$ in the DRG.

*Conclusions*. Our results indicate that abnormal PrP deposition is not uncommonly found in the DRG and nerves of sCJD and dCJD, and that the PrP deposits in the PNS would not correlate with clinical presentation of peripheral neuropathy in CJD.

### 121. DSCR1(Adapt78) Regulates Tau Phosphorylation

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Hyperphosphorylation of the tau protein can lead to formation of neurofibrillary tangles (NFT) that are associated with several neurodegenerative diseases, including Alzheimer's disease (AD). We previously demonstrated that the DSCR1(Adapt78) gene is overexpressed in AD. Here we tested whether DSCR1(Adapt78) overexpression could induce phosphorylation of the tau protein. A DSCR1(Adapt78) transgene was overexpressed in PC-12 cells using the tet-off gene expression system. Induction of DSCR1(Adapt78) caused increased levels of phosphorylated tau protein. DSCR1(Adapt78) has been previously demonstrated to downregulate the activity of calcineurin, a serine/threonine phosphatase (PP2B, which can dephosphorylate tau and might prevent formation of NFTs. Here we show that DSCR1(Adapt78) can also induce production of glycogen synthase kinase-3 beta (also called tau protein kinase I), which is responsible for tau phosphorylation. Thus, DSCR1(Adapt78) which can both down-regulate dephosphorylation of the tau protein (by calcineurin), and up-regulate tau phosphorylation glycogen synthase kinase-3 beta,) may be an important player in neurodegeneration and AD.

### 122. Morphometric Analysis of 3- and 4-Repeat Tau Neuronal and Glial Inclusions in Frontotemporal Dementia (FTD)

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*Background*. Frontotemporal dementia (FTD) is a term describing diverse neuropathological entities presenting with a similar clinical phenotype. Consistent frontal and/or temporal neuronal loss and glial changes are typically observed. However, most descriptions are limited to qualitative observations. By using stereological analysis, we have quantitatively assessed differences in 3-repeat (3R) and 4-repeat (4R) tau protein in neuronal and glial cells in FTD cases.

*Methods*. Formalin-fixed brain specimens from 10 Pick's disease (PiD), 10 corticobasal degeneration (CBD), and 10 familial FTD with exon 10+16 mutation (FFTD) cases were compared with 10 non-diseased controls. Seven-μm sections were taken across brain regions. Immunostaining was performed with antibodies (RD3, RD4) specific for 3R and 4R tau. Cases were then stereologically assessed and a quantitative analysis of pyramidal neurones and glial cell numbers was conducted.

*Results*. 3R tau-positive neurones and glial cells showed the highest expression in PiD and those CBD and FFTD cases with a Pick phenotype. Some FFTD and CBD cases showed significant 4R neuronal and glial reactivity, particularly in instances of concomitant Alzheimer pathology.

*Conclusions*. These antibodies are potentially useful tools in establishing and distinguishing the tau isoform profiles for these disorders and imbalances in the 3R:4R ratio may be a central aetiological mechanism in these tauopathies.

### 123. Frontotemporal Dementia and Parkinsonism Linked to Chromosome 17 (FTDP-17) Associated with the Exon 10 +3 Mutation in the Tau Gene

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*Introduction*. A mutation of the third nucleotide of the intron following exon 10 of the Tau gene is associated with a neurodegenerative disease that has been called multiple system tauopathy with presenile dementia (MSTD). We are studying a family in which 41 individuals across 7 generations have presented clinical symptoms of MSTD.

*Methods*. For this study, we have selected 27 subjects. Fifteen individuals are living, 8 of whom are clinically affected. Twelve subjects are deceased, 10 of whom were clinically affected at the time of death. Neuropathologic studies and immunohistochemistry using anti-tau antibodies were carried out on all 12 of these individuals. Molecular genetic analysis of Tau gene was carried out by direct sequencing.

*Results*. The clinical onset may occur from late in the fourth decade to late in the sixth decade of life. The main neuropathologic changes are the presence of tau deposits in neurons and glia. The neocortex, hypothalamus, midbrain and pontine nuclei are the areas most severely involved. Numerous oligodendroglial cells contained tau deposits. Brain atrophy and neuronal loss vary extensively in relation to the duration of the disease. Molecular genetic analyses showed that the 18 clinically affected individuals were mutation carriers.

*Conclusions*. The present studies extend our knowledge on MSTD and FTDP-17. (P30 AG10133).

### 124. Several Neurodegenerative Diseases of the Brain are Characterized by the Presence of Protein Inclusions

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Tau protein deposits have been found in Alzheimer's disease, Pick's disease, progressive supranuclear palsy, corticobasal degeneration and frontotemporal dementia linked to chromosome 17 (FTDP-17). In FTDP-17 the presence of tau protein deposits is usually associated with mutations in the Tau gene. We have investigated the presence of Tau gene mutations in several familial cases with frontotemporal dementia with or without movement disorders. The genetic screening was correlated with the neuropathological study of brain tissue from some of the patients.

*Methods*. Tau gene exons and exons/intron junctions were sequenced in members of 3 different families with FTD. Brains from members of the families were examined neuropathologically and immunohistochemistry was performed in tissue sections using a panel of antibodies including anti-tau, anti-α-synuclein and anti-ubiquitin antibodies.

*Results*. No mutations were found in the Tau gene. The three families presented different molecular neuropathological features. One family had abundant tau deposits and α-synuclein accumulation only in the Nucleus basalis of Meynerth. A second family presented

abundant ubiquitin inclusions and occasional neurofibrillary tangles while a brain from the third family showed very abundant ubiquitin and tau deposits.

*Conclusions*. Despite clinical similarities families with FTD can present heterogenous neuropathological features. It remains to be investigated whether specific phenotypes are associated with ubiquitin inclusions and the presence of α-synuclein deposits.

### 125. Ubiquitin Pathology Suggests MND, MND-Dementia and FTD-MND Type Represent a Clinicopathological Spectrum

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*Background*. Ubiquitin-immunoreactive (ub-ir) inclusions in motor neurons are characteristic of motor neuron disease (MND). Cases of MND with dementia also have ub-ir neurites and neuronal inclusions in extramotor cortex. A subset of frontotemporal dementia (FTD) patients, without motor involvement, have similar cortical pathology (FTD-MND type). The relationship between these three conditions is unclear.

*Methods*. Ubiquitin immunohistochemistry on tissue sections from numerous anatomic sites from cases of MND without dementia (N = 20), MND-dementia (N = 11), FTD-MND type (N = 15) and controls (N = 10).

*Results*. *i)* Neurites and small dense cytoplasmic inclusions in extramotor cortex in all cases with dementia and 8 of 20 MND without dementia. *ii)* Similar changes in subcortical areas (particularly striatum and thalamus) in all cases with dementia and several MND without dementia. *iii)* Filamentous skeins and Lewy-like bodies (LLB) in motor neurons in almost all cases of MND and in brain stem motor neurons in 4 of 15 FTD-MND (spinal cord tissue not available). *iv)* Skeins and LLB common in substantia nigra and inferior olive in all 3 groups. *v)* Intranuclear neuronal inclusions in 7 of 15 cases of FTD-MND type and 1 of 11 MND-dementia, all with a family history.

*Conclusions*. Several novel patterns of ubiquitin pathology were identified. Overlap in the morphology and anatomic distribution of pathology suggests classical MND, MND-dementia and FTD-MND type represent a clinicopathological spectrum of disease. Intranuclear inclusions may be a specific marker for a subset of familial FTD.

### 126. Is Diffuse Argyrophilic Grain Disease a New Variant of 4-Repeat Tauopathy Different from Limbic Argyrophilic Grain Disease?

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*Background*. Argyrophilic grain disease (AGD) is characterized by the occurrence of argyrophilic grains and coiled bodies in limbic areas located in the temporal lobe. Recent biochemical data have shown that their main components are tau aggregates, corresponding to protein isoforms of 64/69 kDa.

*Methods*. In a series of demented patients who were prospectively followed before death, and whose brain was obtained at autopsy, neuropathological examination, immunohistochemistry and biochemical analysis of tau protein isoforms were performed on brain tissue samples.

*Results*. In 2 of 90 patients, clinical, neuropathological and biochemical investigations clearly demonstrated AGD. Diffuse tau pathology was shown by silver staining, tau immunohistochemistry and tau variant biochemical analysis, not only in temporal lobes but in all cortical and subcortical areas that were assessed. Primary motor, primary sensory, and associative brain cortices were involved, as well as brainstem, but not cerebellum.

*Conclusion*. We suggest that diffuse form of AGD might be a subgroup of 4-repeat tauopathies, which specific profile would be different of that of limbic AGD.

### 127. Sporadic Multiple System Tauopathy with Dementia—An Additional Case

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An 85-year-old woman with 10 years of slowly progressive dementia, presented with "forgetfulness" (MMSE = 27/30) that evolved into difficulty managing finances and recalling recent events. Serial examinations documented progression to probable Alzheimer's disease, although the course was atypical. Parkinsonism, upper motor neuron signs, and family history were absent. MRI showed frontotemporal atrophy. At autopsy, the 536 g hemibrain demonstrated prominent temporal atrophy, and old frontal and parietal infarcts. The neocortex was relatively preserved while limbic-related cortices and medial temporal areas showed prominent changes. Variable numbers of senile plaques (SPs), amyloid angiopathy, small numbers of neurofibrillary tangles (NFTs) (Braak stage II-III) and pretangles were present. The most striking finding was large numbers of tau (PHF1)-positive pleomorphic, coarsely granular glial inclusions. Hippocampal sector CA2 and subiculum showed neuronal loss, gliosis, and few NFTs and SPs, while other sectors were preserved. Marked neuronal loss, gliosis, moderate SPs, few ballooned neurons but no NFTs occurred in amygdala. The nigra, periaqueductal gray and hypothalamus showed cell loss and gliosis. Scattered tau-positive grains also were present, primarily in entorhinal and temporal neocortex. Differential staining revealed the inclusions to be primarily 4R tau. These findings, similar to those of Bigio et al (*JNEN* 60:328-41) and Byrne et al (*JNEN* 61:460), expand further the spectrum of tauopathies.

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### 128. Tau-Positive Neurofibrillary Tangles in an Atypical Case of Hypertrophy of the Inferior Olive

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Hypertrophy of the inferior olive is a transneuronal degeneration commonly seen after lesions in the contralateral dentate nucleus or in the ipsilateral central tegmental tract. In this condition a proliferation of neurofilaments in the inferior olivary nerve cells has been observed by electron microscopy (*JNEN* 30:571-581), but the abnormal nerve cells do not usually give a positive reaction with antibodies to tau (*JNEN* 61:459). Here we present a case of severe atherosclerotic cerebrovascular disease in an 80-year-old man with chronic infarcts involving mainly the left basilar and tegmental pons, where the inferior olives displayed prominent neurofibrillary tangles, readily visible in hematoxylin-eosin stain. The character-

istics of neurofibrillary tangles were confirmed in Bielschowsky silver stain and by immunostaining with 3 different antibodies to tau. By electron microscopy paired helical filaments were demonstrated. α-Synuclein staining was negative. Formation of tau-positive neurofibrillary tangles is an unusual manifestation of transneuronal degeneration, especially in the inferior olive. We have, however, seen it in a case of severe Huntington's disease (*Adv Neurol* 1:453-470) and after longstanding severe basal ganglia infarction (*Acta Neuropathol* 62:96-107).

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## POSTERS

### 130P. An Immunohistochemical Study on Apoptosis in FTDP-17

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*Background*. Frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17) is a genetic disorder characterized by mutation of the tau gene. Although neurofibrillary change accompanied with tau accumulation and neuronal death has been documented as characteristic neuropathological events, a little is known about the relationship between them. Since the neuropathological change found in FTDP-17 is independent of Ab, it would be useful to know if accumulation of tau is sufficient for neurodegeneration and apoptosis in tauopathy.

*Methods*. Two cases of FTDP-17 (S305N and N279K) were examined. We used tau, Bax and Bcl-2 immunohistochemistry in the hippocampus and parahippocampal cortex from patients with FTDP-17, Alzheimer disease (AD) and normal healthy controls. TUNEL was performed on tissue sections using an ApopTag (Intergen Company, Purchase, NY).

*Results*. In the case of S305N, tau-positive neurons were most prominent in frontal, temporal, insular and postcentral cortices, as well as in the dentate gyrus. Case N279K showed intense tau deposition in the medial temporal cortices and upper and lower motor neurons. TUNEL-positive neurons were observed frequently in tau-positive neurons. Bax and Bcl-2 upregulations were seen in tau-negative neurons. The findings suggest that tau accumulation is accompanied by DNA fragmentation.

### 131P. A Clinicopathological Study of Frontotemporal Dementia: Our 5 Autopsied Cases

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Frontotemporal dementia is a relatively common form of dementia among non-Alzheimer-type dementia. Recently, they were classified into three subgroups. Nosology of these dementia still remains to be elucidated. We have an opportunity to examine 5 cases of these dementia by clinical and neuropathological, and immunopathological viewpoints. All of 5 cases began to show behavioural disorders, inadequate social behaviors, on ages ranging from 27- to 54-years-old, insidiously followed by hyperoralities, compulsive, stereotyped, preservative behaviors. Four cases exhibited unique speech disorders, terminated after 6 to 23 years. The brains appeared atrophic in frontal and temporal lobes with variable degree. There were loss of neurons in the cortex with reactive gliosis. No Pick bodies and Lewy bodies were recognizable. Immunopathological studies usin anti ubiquitin and tau antibodies showed that ubiquitine positive but tau negative cytoplasmic structures in neurons were observed in 3 cases. At present, it would be rational that our 5 cases were primarily fronto-temporal degeneration type, but immunopathological findings might implicate there are still heterogeniety in this disease group.

### 132P. Pontocerebellar Atrophy in Progressive Supranuclear Palsy and Diffuse Lewy Body Disease

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Progressive supranuclear palsy (PSP) and diffuse Lewy body disease (DLBD) show parkinsonism and each pathologic changes have been well delineated. We report 2 patients with PSP and one patient with DLBD in whom atrophy of the pontocerebellar system was seen. The age at death was 71 and 73 in the PSP patients, and was 64 in the DLBD patient. One of the PSP patients showed ataxia. The sections from the paraffin-embedded blocks of the brains were stained with hematoxylin-eosin, Klüver-Barrera, Bodian, and Gallyas-Braak silver stains and were immunostained using the antibodies to tau, α-synuclein and ubiquitin.

Pathologic examination showed loss of myelinated fibers and gliosis in the transverse fibers of the pons and the cerebellar white matter, and neuronal loss with gliosis in the pontine nuclei in addition to the classical lesions of PSP or DLBD in these 3 patients. The inferior olivary nuclei were mildly affected. Argyrophilic threads were seen with Gallyas-Braak silver stain in the inferior olivary nucleus of the DLBD patient. Glial cytoplasmic inclusions were not seen in the brains. We conclude the degeneration of the pontocerebellar system in these patients represents extended lesion of PSP or DLBD , but not concomitant occurrence of multiple system atrophy.

### 133P. Clinicopathological Study of Atypical Progressive Supranuclear Palsy Cases

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Progressive supranuclear palsy (PSP), one of the tauopathy, is characterized by typical clinical features such as postural instability and falls, gaze palsy, and pseudobulbar palsy, but infrequently presents with uncommon features such as pure akinesia, dementia, and absence of gaze palsy. To clarify the pathological basis for the clinical manifestation of PSP, we examined the brains from ten PSP cases presenting with uncommon features in the course (4 cases with pure akinesia, 2 cases with non-fluent aphasia, 2 cases with dementia, and 2 cases with behavioral abnormality). Although all cases showed that argyrophilic, tau-positive neurofibrillary tangles and glial inclusions were found in the basal ganglia and brainstem in distribution consistent with PSP, those pathological changes had a tendency to be accentuated in the basal ganglia in the cases with pure akinesia, in the frontal cortex in the cases with apha-

sia and dementia, and in the medial side of the temporal lobe in the cases with dementia and behavioral abnormality associated with or without amyloid deposition. Our result suggested that the clinical presentation of PSP could be influenced by the various combination in the distribution and severity of subcortical and cortical tau pathology and modified by the co-existing Alzheimer change especially in elderly cases.

### 134P. Neuronal Intranuclear Inclusions Distinguish Familial FTD-MND Type From Sporadic Cases

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University of British Columbia, Canada.

*Background*. Ubiquitin-immunoreactive (ub-ir) neuronal cytoplasmic inclusions are characteristically found in extramotor cortex in patients with motor neuron disease and dementia (MND-dementia) and a subset of patients with frontotemporal dementia without motor symptoms (FTD-MND type). Recently, ub-ir neuronal intranuclear inclusions have been described in a small number of patients with familial FTD-MND type.

*Method*. To better define the sensitivity and specificity of this pathological change, we examined post mortem tissue from 14 patients with FTD-MND type (9 familial, 5 sporadic), 11 cases of MND-dementia ( 5 familial, 6 sporadic) and 19 cases of MND with no history of cognitive dysfunction (2 familial, 17 sporadic).

*Results*. Numerous intranuclear inclusions were found in multiple anatomic sites in 7 of 9 cases of familial FTD-MND. Rare intranuclear inclusions were present in the hippocampal dentate granule cells in one case of familial MND-dementia. No sporadic cases had intranuclear inclusions.

*Conclusions*. These findings suggest that intranuclear inclusions are specific for familial FTD and may identify a subset of families with a common molecular pathogenesis. Although intranuclear inclusions are most characteristic of families in which the clinical presentation is pure FTD, they may also be found in some pedigrees with both FTD and MND; further supporting the hypothesis that FTD-MND type and MND-dementia represent a clinicopathological spectrum of disease.

### 135P. Idiopathic Parkinson's Disease with Motor Neuron Disease

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Combined parkinsonism, dementia and motor neuron disease (MND) are features of frontotemporal dementia with parkinsonism linked to chromosome 17q (FTDP 17-q), ALS/parkinsonism dementia complex of Guam (ALS/PDC) and frontotemporal dementia with motor neuron disease (FTD-MND). Pathological findings in FTDP-17q and ALS/PDC are characterized by deposition of neuronal and glial insoluble tau protein while ubiquitin positive (tau and α-synuclein negative) pathology characterizes FTD-MND. We report 2 cases of sporadic idiopathic Parkinson's disease (iPD) with MND. Both case had rest tremor, rigidity, bradykinesia, fasciculation and other features consistent with extrapyramidal and motor neuron dysfunction. Both cases had EMG evidence of diffuse lower motor neuron disease. Pathological analysis in both cases demonstrated substantia nigra pallor, anterior horn cell loss, Bunina bodies, alpha-synuclein positive Lewy bodies in brainstem and limbic cortex and ubiquitin positive (α-synuclein negative) inclusions in motor neurons consistent with mixed Lewy body disease (LBD) and MND. These cases demonstrate that mixed LBD and MND occur, and must be considered as a diagnostic possibility in cases presenting as mixed iPD and MND.

### 136P. Novel Tau-Positive Fine Granules in the Cerebral White Matter of Tauopathies

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We report a novel neuropathological finding, tau positive fine granules (TFGs), in the cerebral white mater of the parkinsonism-dementia complex of Guam (PDC). Brains with PDC, Guamanian controls, Japanese CBD, PSP, Pick's disease, Alzheimer's disease (AD) and myotonic dystrophy (MyD) , were examined immunohistochemically and biochemically using anti-tau antibodies. TFGs were tau positive, TFGs were globe-shaped and 3 to 6 μm, in size. Confocal analysis revealed an absence of GFAP and neurofilament in TFGs, and immunoelectron microscopic study suggested that TFGs were not identical to argyrophilic threads and inseparably connected with outer layer of myelin sheath and oligodendroglial cell bodies. TFGs were predominantly observed in the frontal white matter in 30 PDC patients out of 35. However, no TFGs were found in the brains of PSP, MyD, Pick's disease, AD, or CBD. Western blot analysis of the cerebral white matter in PDC showed sarkosyl-insoluble tau composed of two major bands of 60 and 64 kd with a minor band of 68 kd, and 4R-tau isoform accumulation. Conclusively, TFGs were exclusively found in PDC brains, novel finding in human brain, and a specific neuropathological marker of PDC. In addition, this study revealed accumulation of 2 types of tau isoforms in one brain.

### 137P. Elimination of NFT/PHF in the Parkinsonism-Dementia Complex of Guam: Obserevation in Human and Inoculated Rat Brains

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Neurofibrillary tangles (NFTs) or paired helical filaments (PHFs) composed of 6 isoforms of tau protein have been reported to be hardly dissolved, and sustained for a long time in the brain with Alzheimer disease. In the present study, to ascertain whether or not NFTs of parkinsonism-dementia complex (PDC) of Guam, also composed of the 6 isoforms, are actually persistent in brain, we performed 2 ways of research. *i)* A quantitative study of NFTs and neurons in the CA1 of the temporal lobe in PDC. *ii)* Chronological obser-

vation of CA1 of rat brain after inoculation of the NFTs/PHFs extracted from PDC.

*Results*. *i)* Evidence of the disappearance of NFTs was observed in the patients with PDC. *ii)* Inoculated NFTs/PHFs were tau-immunopositive clusters 3 days and one week after inoculation. After 2 weeks, tau-positive granules were found in so-called Gitter cells, and after 6 weeks the size of the tau-positive clusters decreased.

*Conclusion*. NFTs/PHFs composed of tau protein with 6 isoforms are eliminated in human and rat brain in PDC.

### 138P. Comparison of Neurofibrillary Degeneration Distribution in DM2 and DM1 Brains

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*Background*. Neurofibrillary degeneration (NFD) is present in the brain of patients affected by Steinert's myotonic dystrophy (DM1). The number of tau protein isoforms is abnormally reduced in DM1 brains. Tau pathology has not been reported yet in DM2 (PROMM).

*Methods*. Brain tissue samples of one DM2 and two DM1 patients, genetically confirmed, were studied by immunohistochemistry.

*Results*. NFD was observed in the hippocampus, the frontal cortex, and the occulomotor motor nuclei. In DM2 patient, the highest NFD density was found in the locus niger, anti-ubiquitin disclosed a numerous intranuclear, Marinesco-like inclusions, without neuron loss or synucleinopathy. NFD was also observed in the anterior horn of the spinal cord.

NFD was immunoreactive for several anti-tau antibodies raised against abnormally phosphorylated epitopes located outside sequences encoded by exons 2 and 3 of the tau gene. Conversely, there was a dramatic loss of immunoreactivity for anti E2 and anti E3 antibodies.

*Conclusion*. NFD is also observed in DM2. The topographic distribution of the NFD closely resembles that of DM1 individuals. Immunohistochemistry strongly suggests that this tau pathology does not result from cerebral ageing or other neurological causative factors. Further analyses would be necessary to determine whether the tau isoform expression if affected similarly to that of DM1 individuals.

## PLATFORM PRESENTATIONS

### 139. Clinico-pathologic Studies in Fasciitis: Diffuse Fasciitis with Eosinophilia and Macrophagic Myofasciitis

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*Objective*. Diffuse fasciitis with eosinophilia (DFE) and macrophagic myofasciitis (MMF) are very rare diseases involved in the fascia of skeletal muscle. We investigated clinico-pathologic studies in thesediseases.

*Patients and methods*. Two cases of DFE and 2 of MMF were performed muscle biopsy. Muscle specimens were stained routine histochemical procedures and immunohistochemical methods.

*Results. i) DFE. Case 1*. A 67-year-old man had a spontaneous pain of shoulder and upper arms and flexion contracture of elbows and knees. His subcutaneous tissues in the upper and lower extremities, shoulders, and back are firm and thickened. *Case 2*. A 52-year-old woman had firm subcutaneous tissue in the lower extremities. In both patients, muscle biopsy showed thickened fascia extending from the subcutaneous tissue to muscle but no inflammatory changes were detected in muscle fibers. *ii) MMF. Case 1*. A 50-year-old woman had slowly progressive weakness of upper and lower extremities before one year. *Case 2*. A 27-year-old woman, who suffered from rheumatoid artheritis, had myalgia and firm subcutaneous tissue in the trunk and four limbs. In both patients, muscle biopsy showed inflammatory infiltrates contained macrophages in muscle and fascia.

*Discussion*. In DFE biopsy disclose thickened fascia extending from the subcutaneous tissue to muscle. Whereas in MMF, muscle biopsy shows macrophagic infiltration of the fascia and endomysium.

### 140. Frequent LOH at Chromosome 12q22-23 and Apaf-1 Inactivation in Glioblastoma

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Apaf-1, the apoptotic protease activating factor-1, located at chromosome 12q22-23, is a major effecter of the p53 mediated apoptosis pathway, and Apaf-1 inactivation due to chromosome 12q22-23 LOH and hypermethylation may be involved in some of the neoplasms in malignancy. However, little is known about the frequency of the 12q22-23 LOH or the state of Apaf-1 in GB. To elucidate their involvement in GB, we analyzed a series of 33 GBs for chromosome 12q22-23 LOH, Apaf-1 mRNA expression, and Apaf-1 protein expression, using microsatellite analysis, RT-PCR analysis, and immunohistochemical (IHC) analysis, respectively. We also evaluated if and how the 12q22-23 LOH correlated with the p53 gene mutation and EGFR gene amplification. Chromosome 12q22-23LOH was detected in 14 (42%) of 33 cases. Among the examined cases with LOH at 12q22-23, a low expression of Apaf-1 mRNA was detected in 9 (69%) of 13 cases, and a low expression of Apaf-1 protein was detected in 12 (86%) of 14 cases. The 12q22-23 LOH was significantly correlated with low expression of mRNA and protein ($p<0.05$, $p<0.001$ respectively). The p53 gene mutation and EGFR gene amplification were found in 13 cases (39%) and 8 cases (24%), respectively, and these gene alterations were inversely correlated. However, 12q22-23 LOH had no correlations with the p53 gene mutation or EGFR gene amplification. Six (67%) of 9 GBs with neither p53 gene mutation nor EGFR gene amplification tested positive for 12q22-23 LOH. These GBs are likely to belong to another subset independent from the two common genetic subsets in GB.Twenty-three out of the 33 GBs (70%) with the 12q22-23 LOH also tested positive for Apaf-1 inactivation or p53 gene mutation. This high frequency of alterations in the apoptosis-associated factors prompts a speculation that abrogation of the Apaf-1 and p53 mediated apoptosis pathway may play an important role in the tumorigenesis of GB.

### 141. Overexpression of the ATP-binding Cassette Half-transporter, ABCG2 (MXR/BCRP/ABCP1), in the Human Malignant Brain Tumors

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*Object*. The ATP-binding cassette proteins constitute a superfamily of transporter proteins, a subset of which has been implicated in multi-drug resistance. The recent expansion in the number of transporters identified has prompted renewed interest in the role of drug transporters in the clinical drug resistance. These newly identified transporters include a half-transporter, namely, MXR/BCRP/ABCP1 (ABCG2). MXR confers high levels of resistance to mitoxantrone, anthracyclines and camptothecines. MXR localizes to the plasma membrane in the cells that highly overexpress the protein either through gene amplification or through gene rearrangement.

*Material and methods*. In the present study, using RT-PCR and monoclonal antibodies of MXR, we investigated MXR in the human malignant brain tumor specimens (13 glioblastoma, 15 anaplastic astrocytoma, 9 diffuse astocytoma, 12 malignant lymphoma, and 1 choriocarcinoma).

*Results*. We demonstrated the presence of MXR at the blood-brain barrier. RT-PCR analysis confirmed the expression of MXR in 9 glioblastoma, 9 anaplastic astrocytoma, 6 diffuse astocytoma. Moreover, we picked up MDR-1, MRP, and MXR as drug-resistant genes and measured the expression of these genes. To achieve a good response to the drugs, we chose anticancer drugs for each patient individually, based on the results of drug-resistant gene expression.

*Conclusion*. Measuring the expression of drug-resistant genes facilitates rapid determination of drug-sensitivity to chemotherapy in the patients with malignant brain tumors.

### 142. Tumor Necrosis Factor-related Apoptosis Inducing Ligand Induces Glioma Cell Proliferation that is Dependent on ERK1/2 and Survivin

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*Introduction*. Tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL/Apo2L) is a member of the TNF family that induces apoptosis by ligation of death receptor 4 (DR4/TRAIL-R1)

and DR5 (TRAIL-R2). Recently TRAIL has attracted a great deal of attention as an anti-cancer agent as it has been found to induce apoptosis in a broad range of transformed cells but has little cytotoxicity to normal cells. Here, we report that TRAIL triggers proliferative signals.

*Results*. We examined nine human glioma cell lines for their responses to TRAIL. TRAIL induced apoptosis in three cell lines, no response in one cell line, and induced a proliferative signals in the remaining five. Proliferation of glioma cell lines was confirmed using crystal violet assay for increased cell numbers, cell cycle analysis by propidium iodide staining and flow cytometry, and retinoblastoma protein phosphorylation by Western blot. Glioma cell lines having proliferative responses to TRAIL showed concomittant ERK1/2 activation as evidenced by its phosphorylation and its ability to phosphorylate the Elk-1 transcription factor. TRAIL treatment increased survivin expression ten fold in glioma cells in which it induced a proliferative response, but not in cells sensitive to TRAIL-induced apoptosis. TRAIL-mediated glioma cell line proliferation and the increase in survivin level were abolished by MEK inhibitor treatment.

*Conclusions*. The results indicate that TRAIL can induce both apoptotic and proliferative responses in glioma cell lines. TRAIL-mediated proliferation in glioma cells is dependent on ERK1/2 activation and by an increase in survivin expression

## PLATFORM PRESENTATIONS

### 143. Antisense VEGF Oligonucleotides (ODN) Vehiculated by Solid Lipid Nanoparticles (SLN): In Vitro and In Vivo Study in an Animal Model of Cerebral Glioma

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*Objectives*. To evaluate if VEGF antisense oligonucleotides carried by SLNs downregulate VEGF expression in vitro and in vivo in an experimental model of rat brain glioma.

*Methods*. C6 glioma cells in standard and hypoxic conditions were treated with antisense rat VEGF exon 3 ODNs administered as free solutions (10 to 100 mcM) and vehiculated by SLN (ODN-SLN) (100 nM). VEGF mRNA and protein expression was evaluated by means rt-PCR and Western blot semi quantitative analysis. C6 glioma cells were implanted using stereotactic technique in rat brain; after 13 days animals were systemically treated with ODN-SLNs or with SLN alone. Brain coronal sections were obtained after 2 to 4 days and ODN tissue distribution, VEGF expression and tumor mass volume were evaluated.

*Results*. In vitro after treatment with ODN-SLNs VEGF expression was remarkably reduced compared with ODN free solutions, both in hypoxic and in standard conditions. In vivo ODN-SLNs are able to reach the tumor at higher concentrations compared to the surrounding brain tissue and to modulate VEGF expression.

*Conclusions*. ODN-SLNs are able to modulate VEGF expression both in vitro and in vivo and to reach and reduce the tumor mass suggesting a potential role in human malignant glioma therapy.

### 144. HIFα and VEGF in Diffuse Astrocytomas: Prognostic Implications

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*Purpose*. Hypoxia-inducible factor (HIF)-1α is a transcription factor that promotes ischemia-driven angiogenesis. The aim of this study was to determine the relation of HIF-1α to vascular endothelial growth factor (VEGF), p53 expression, angiogenesis, proliferative potential and clinical outcome in a large series of diffuse astrocytomas.

*Experimental design*. Expression of HIF-1α, VEGF, Ki-67 (a proliferation associated marker) and p53 was determined immunohistochemically in 83 adult patients with supratentorial diffuse astrocytomas. Microvessels, highlighted by means of anti-CD34 immunohistochemistry, were enumerated with computer-assisted image analysis. Univariate and multivariate survival analyses were performed to test the prognostic significance of HIF-1α.

*Results*. Although HIF-1á and VEGF were expressed in the majority of cases, their levels increased significantly with increasing grade and proliferative potential. HIF-1α positively correlated with microvessel counts and VEGF with total vascular area and the presence of rounder vessel sections. There was a positive correlation of VEGF with p53 expression in astrocytomas and anaplastic astrocytomas. In univariate analysis, both VEGF and HIF-1α were associated with shortened survival in the entire cohort, but lost significance when grades II/III and grade IV were analyzed separately. Multivariate analysis revealed that the interaction of HIF-1α with grade was a significant prognostic indicator.

*Conclusions*. HIF-1α expression may be used to refine the prognostic information provided by grade in patients with diffuse astrocytomas. Its adverse prognostic effect is most likely mediated by hypoxia, the driving force for HIF-1α accumulation.

### 145. Significance of Macrophage Infiltration-associated Thymidine Phosphorylase Expression in Glioma: Correlations with Angiogenesis and Poor Prognosis

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*Background*. Angiogenic enzyme thymidine phosphorylase (TP) has been implicated as a potent angiogenic factor and an indicator of poor prognosis in various solid tumors. The significance of TP in glioma remains unclear because TP is also known as gliostatin, a selective growth inhibitor against all glial cells.

*Methods*. TP expression was immunohistochemically examined in a series of 50 astrocytic tumors with different malignancy and correlated with tumor angiogenesis and apoptosis, as well as prognosis. To identify the histological type of TP-positive cells, a further immunostaining TP together with CD68, a macrophage marker, or with GFAP on several mirror sections was performed.

*Results*. The majority of TP-positive cells were of macrophage origin. TP expression was significantly associated with glioma malignancy grading, intratumoral microvessel density, and vascular endothelial growth factor (VEGF) expression but showed no relationship with apoptotic index or p53 expression. Regardless of glioma grading, patients with TP-positive tumors had a significantly shorter mean survival time than those with TP-negative tumors.

*Conclusions*. These findings suggest that TP might play a crucial role in angiogenesis during glioma development, and immunodetection of TP is useful for clinical prediction. Further studies are necessary to better elucidate the role of TP in glioma, which may provide insights into adequate TP-targeted therapy.

### 146. Vascular Response in the Astrocytic Tumors. Immunohistochemical Study of the Factor-VIII-Reagent, Alpha Smooth-Muscle Actin, Laminin, Tenascin, and Fibronectina

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*Introduction*. Astrocytic tumors are the most frequent intracranial neoplasms. One of the most characteristics malignant features is the vascular proliferation.

*Objective*. The aim of this work was to study the vascular response in the astrocytic neoplasms, its cellular and extracellular matrix components; to analyze the relationship between vascular response, and extracelular matrix components.

*Methodology*. Surgical specimens from 68 human glioma biopsies were revised, and only 38 was used. Immunohistochemical stain-

ing for GPAP, vimentin, Factor-VIII-Reagent (FVIII-Rag), alpha smooth-muscle actin (α-SMSA), laminin, tenascin, and fibronectin was performed.

*Results*. The vascular response was diverse and was related to malignant grade. The cellular component showed positive staining for FVIII-Rag and α-SMA, even in the intimal layer. Positive immunostaining for laminin, tenascin and fibronectin was observed in the "glomeruloid" vessel and in the vessels with thickened walls. The tenascin was also positive in some case of pylocitic astrocytoma, and laminin was excellent detecting of the glial and endothelial basement membrane.

*Conclusion*. The vascular response was related with the malignant grade and was better viewed with the use of extracellular matrix markers. The α-SMA had limited value in the identification of the cellular component. The extracellular matrix markers had no relationship with the malignant grade. The external limiting glia could be responsible for the "glomeruloid" aspect of the vascular response.

### 147. A Morphological Spectrum of Neoangiogenesis in Childhood Brain Tumors, Cortical Tuber and Rassmusen's Encephalitis

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More than 3 decades ago, J. Folkman introduced the hypothesis that the survival and growth of most solid tumors is dependent on proper blood supply by the newly-formed tumor vasculature. Since that time, it has become apparent that, in addition to solid tumors, numerous other systemic and CNS non-neoplastic diseases are also "angiogenesis-dependent" disorders. Conventional wisdom holds that postnatally formed tumor-related and other blood vessels are morphologically simple endothelial-cell lined channels with or without added endothelial-smooth muscle cells haphazardly disposed along the outer border of a vessel wall. On the other hand, various morphological-functional subtypes of vascular malformations of the CNS are presumed to be congenital disorders of mesodermal differentiation occurring during the first trimester of gestation. However, it has been repeatedly observed that various subtypes of CNS vascular mlformations may coexist with one another or with a brain tumor. There are also well documented rare case reports of "de novo" developing cavernous angioma (CA), as well as arteriovenous malformation (AVM) "induced" by brain tumors expressing vascular endothelial growth factor (VEGF).

Our review of 120 brain tumors surgically removed at our institution over the past 4 years revealed that 6 (5%) were associated with either an AVM (2) or CA (4). We have also demonstrated a peculiar neonangiogenesis in the cortical tuber surgically removed from a patient with TS, and in the cortex of a patient with Rasmussen's encephalitis. We believe that these and previously reported human cases support experimental evidence that angiogenesis may have a pathogenetic role in the development of various vascular malformations of the CNS.

## PLATFORM PRESENTATION

### 148. Degeneration of Neurites Within "Cotton Wool" Plaques in Variant Alzheimer's Disease Caused by Deletions of the Presenilin 1 Gene

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*Introduction*. In familial Alzheimer's disease (FAD) production of Aβ42 is elevated and—according to the amyloid cascade hypothesis—its deposition in brain is of primary pathoegenetic importance (Hardy and Selkoe, *Science* 297: 353). The toxic effect of nonfibrillar Aβ deposits ("cotton wool" plaques; CWPs) was examined by quantifying the density of neurites within and around CWPs in the variant FAD with spastic paraparesis due to deletion of either amino acids 83 and 84 (DI83/DM84) or exon 9 (Dexon9) of presenilin1. Aβ42 burden was also measured.

*Materials and methods*. Sections of one DI83/DM84 and 4 Dexon9 patients' brains were examined after myelin staining and immunostaining for Aβ42 as well as by confocal microscopy after double immunostaining for Aβ42 and neurofilament (NF) or hyperphosphorylated tau (h-tau).

*Results*. The densities of myelinated axons and NF+ neurites within CWPs were significantly lower than in the surrounding parenchyme ($p<0.01$), whereas the density of h-tau+ neurites (neuropil threads) was higher ($p<0.01$). The density of neurites alongside CWPs did not differ from that above/below CWPs. The Aβ burden in Dexon9 patients was greatest in motor cortex representing lower extremities.

*Conclusion*. Nonfibrillar Aβ in CWPs is neurotoxic and might be responsible for the development of dementia and paraparesis in variant FAD with CWPs.

### 149. Possible Regional Correlation of NFT and CAA Lesions in the Hippocampus of AD Brains

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The discrete and focal distribution of neurofibrillary tangles (NFTs) in the brains of victims of Alzheimer's disease (AD) is an important but poorly understood characteristic of this dementing illness. Additionally, the very high coincidence of congophilic amyloid angiopathy (CAA) in many of these patients may also be significant. In this study the distribution and frequency of these two lesions were examined throughout the hippocampal formation of control and Alzheimer patients in order to determine whether any regional correlation existed. Serial coronal sections through the entire left hippocampal formation of 6 controls and 6 Alzheimer brains were stained with Congo red for light-microscopic examination under polarized illumination. The neuronal, extracellular and vascular frequency and distribution of apple green birefringent material was recorded. In control brains there was an even anteroposterior distribution of tangles and comparatively few and evenly distributed amyloid positive intracortical vessels. However, in the anterior coronal half of the hippocampus of Alzheimer brains there appeared to be a propensity for intraneuronal NFT distribution and increased vascular deposition of amyloid material. In the AD cases, the NFTs sometimes appeared in clusters in discrete regions. These results support the view that some spatial correlation may exist between tangles and amyloid-positive vessels in specific regions of the human hippocampus in dementia of the Alzheimer type.

### 150. Alzheimer (AD) Pathology in the Brainstem of Non-demented (ND) Subjects

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Some poorly understood manifestations in AD may be interpreted from the perspective of brainstem dysfunction. Significant pathology in this region has been documented in AD, but no report has had the primary aim of examining the status of the brainstem in the ND subjects. We provide a preliminary examination of this region in a series of patients with no previous neurological or psychiatric history.

The prevalence, distribution and severity of the AD changes in the brainstem were studied in 36 ND subjects (mean age 66) and compared to a series of 22 age-matched AD brains. Transversal sections at four levels were immunostained with commercially available anti-tau and anti-amyloid antibodies. A scale of 0 (absence) to 3+ (high accumulation) was applied to data for a semi-quantitative estimate of changes.

In addition to cerebral involvement, senile plaques, tau pathology, or both, were present in the brainstem of 36% of the ND and in 95% of the AD cases. Sparse diffuse plaques and very occasional tau+ cells (neuronal and glial) were observed in the ND group in contrast to a moderate-to-high accumulation in the AD brainstems. A rostral-caudal gradient was found in both groups.

We conclude that, similar to cortical findings, very mild AD changes are not uncommon in the brainstem of ND ageing subjects. This study suggests that an accurate neuropathological evaluation of the brainstem would be of interest in ageing, because of the important consequences in neuroscience research.

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### 151. Immunohistochemical Study of RAGE Expression in Alzheimer's Disease and Control Brain Samples

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*Introduction*. The abnormal accumulation of the β-amyloid peptide is one of the key factors for the diagnosis of Alzheimer Disease (AD). The receptor of the advanced glycation and products (RAGE) is the unique β-amyloid receptor totally characterized and studied in detail. This molecule, included in the superfamily of the immunoglobulins has also receptor functions of the advanced glycation and products (AGEs). Normally this receptor expresses itself in the microglia, the endothelial cells and certain neuronal forms. The expression of RAGE can suppose a risk factor for the neurons exposed to abnormal concentrations of the β-amyloid peptide (as this is the case of the DA) or of AGEs (as this is the case of ageing).

*Methods*. In order to recognize neuronal and glial population vulnerable for β-amyloid toxicity, 10 brains of patients with the diagnosis of AD and 4 controls of young age and 11 enderly persons, have

been studied immunohistochemically for the RAGE, as well as for β-amyloid, tau, GFAP and CD68 in simple and double tinctions of the hippocampus, enthorrinal cortex, frontal and parietal cortex with the objective to analize the expression of RAGE as possible pathological indicator in neuronal populations susceptible during aging and AD.

*Results*. RAGE is overexpressing in the AD cases point to the neuronal oxidative stress.

### 152. Gene Expression in APP Intracellular Domain (AICD) Overexpressing Human Neuroblastoma Cells: Implications for Alzheimer's Disease

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Processing of the beta-amyloid precursor protein (APP) by the beta- and gamma-secretases leads to the production of 2 small peptides, the Alzheimer's disease (AD)-associated amyloid beta-peptide (Abeta) and the APP intracellular domain (AICD). Whereas the role of Abeta in the pathogenesis of AD has been studied extensively, the role of the small AICD protein (mostly 50 or 57 aa long) is yet poorly understood. AICD was originally shown to lower the cellular threshold to apoptosis and more recently to modulate gene expression. In order to elucidate target genes of AICD (resp. an AICD transcription complex) we cloned the 2 most favoured (due to gamma-secretase processing) AICD isoforms (AICD50 and AICD57) in different expression vectors. Further we generated human neuroblastoma cell lines (SHEP-SF) stable expressing each of the two AICD isoforms. Here we report the results of a microarray study comparing AICD overexpressing cells versus mock vector stable transfected cells. We used Affymetrix U133 GeneChips, which comprise more than 22 000 genes.

### 153. PDAPP Alzheimer Transgenic Mice: Ab-associated Neuritic Changes in the Grey and White Matter

Capobianco R; Giaccone G; Mangieri M; Morbin M; Mazzoleni G; Kingston AE; Tagliavini F; Bugiani O

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*Background*. Transgenic mice overexpressing human amyloid precursor protein (APP) with the familial Alzheimer V717F mutation (PDAPP mice) have been shown to develop abundant cerebral Aβ protein deposits in an age-specific and brain region-specific manner. Although they are not associated with neurofibrillary pathology, Aβ deposits in PDAPP mice are similar to those of Alzheimer disease (AD) in inducing a local tissue damage marked by astrogliosis, microglia activation and neuritic alterations.

*Methods*. To better characterise these changes, we have analysed the antigenic profile of neurites associated with Aβ deposits in 4- and 12-month-old PDAPP mice by immunohistochemistry with antibodies to Aβ, APP, ubiquitin, synaptophysin, α-synuclein, MAP2, phosphorylated and non-phosphorylated neurofilaments.

*Results*. Aβ deposits were surrounded by and intermingled with dilated neuronal processes strongly labeled by antibodies to APP, synaptophysin and ubiquitin not only in the hippocampus and in the cerebral cortex, but also in the corpus callosum and external capsule. At the ultrastructural examination, these neurites were filled with multilamellar bodies and the cytoskeletal components were displaced to the periphery.

*Conclusions*. These results demonstrate that the development of ubiquitin- and APP-loaded cellular profiles associated with Aβ deposits is tightly related to the appearance of aberrant synaptic terminal formation and may either precede or occur independently from neurofibrillary changes in the morphogenesis of senile plaques.

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## POSTERS

### 154P. Plasma Levels of β-amyloid 42 are Increased in Women with Mild Cognitive Impairment

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[3] Department of Neurology, Valloria Hospital, Savona, Italy.

*Background*. Accumulation in the brain of small, soluble aggregates of amyloid β-protein 42 (Aβ42) is the major pathogenic event of Alzheimer's disease (AD). In familial early-onset AD this event is likely the result of Aβ42 overproduction, in the most common sporadic late-onset form of the disease the mechanisms of Aβ42 accumulation are unknown.

*Methods*. To begin to address this issue we analyzed plasma levels of Aβ40 and Aβ42 in 64 elderly patients with mild cognitive impairment (MCI), chosen as paradigm of preclinical AD.

*Results*. We found a significant increase of Aβ42 plasma levels in women with MCI, in comparison to the affected males and to 64 cognitively normal age-matched subjects. Aβ42 plasma concentrations were not correlated with apoE genotype, cholesterol and creatinine plasma levels.

*Conclusions*. These findings represent the biological explanation for the gender-dependent increased incidence of late-onset AD in women reported by epidemiological studies. They also indicate that sporadic late-onset AD in women is due to a primary Aβ42 overproduction that is likely to depend on postmenopausal estrogens deprivation.

### 155P. Clinical and Neuropathologic Phenotype of a Large Italian Alzheimer Family with Presenilin 2 Mutation M239V

Marcon G; Giaccone G; Mazzoleni G; Cupidi C; Spinello S; Finato N; Beltrami CA; Bergonzi P; Sorbi S; Tagliavini F; Bugiani O

Milano, Italy; Udine, Italy; Firenze, Italy.

*Background*. Presenilin 1 and 2 are two highly homologous genes involved in familial Alzheimer's disease (FAD). While more than 100 mutations in presenilin 1 are known to segregate with the disease in FAD, only 9 mutations in presenilin 2 (PSEN2) have been identified to date.

*Methods*. We report the clinical and neuropathologic phenotype of FLO10, the large Italian FAD kindred associated with methionine to valine substitution at residue 239 of PSEN2.

*Results*. The patients showed a remarkable variability in age of onset of symptoms (45-83 years), disease duration (4-22 years) and clinical presentation. The neuropathologic analysis of 2

patients revealed peculiar features in addition to the Alzheimer hallmark lesions neurofibrillary changes and A amyloid deposits. Ectopic neurons in the subcortical white matter, often containing neurofibrillary tangles, were found in both patients, one of whom presented with epilepsy. Another finding was the presence in one of the patients of an unusually high number of ghost tangles diffuse in the cerebral cortex and exhibiting immunoreactivity for the 40-residue form of Aβ.

*Conclusions*. The results of this study confirm that the FAD kindred FLO10 associated with M239V mutation of PSEN2 is characterised by some peculiarities of the clinical and neuropathologic phenotype compared to sporadic Alzheimer's disease.

### 156P. Characterization of CLAC-positive Senile Plaques in Alzheimer Brains

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University of Tokyo, Japan; University of Manchester, United Kingdom.

We identified CLAC (collagen-like Alzheimer amyloid plaque component) as a novel constituent of senile plaques (SP). Primitive SP as well as the periphery of typical SP in the cerebral cortices of patients with Alzheimer's disease (AD) were CLAC-positive, whereas diffuse SP and cerebrovascular amyloid deposits were negative. We found that thioflavin S reactivity of SP amyloid was inversely correlated with the positive reaction for CLAC. Immunoelectron microscopy showed that CLAC-positive bundles were more loosely packed and less electron-dense compared with CLAC-negative bundles, which was consistent with the complementary distribution of CLAC-positive and thioflavin S-positive deposits. We next addressed the question whether CLAC contributes to the protease-resistance of amyloid deposits. Paraffin sections from AD cortices fixed in 10% paraformaldehyde for 24 hours were treated with proteinase K and then immunostained for Ab or CLAC. We found that Aβ42-immunoreactivities were preserved in the CLAC-positive SP, whereas those negative for CLAC, including cores of typical SP harboring abundant amyloid deposits, lost immunoreactivities for Aβ C termini after proteinase K digestion. In vitro studies have suggested that CLAC may inhibit fibrillization of Aβ, acting against the deposition of SP amyloid. In contrast, our protease digestion studies suggested that CLAC may confer protease-resistance to amyloid fibrils, especially to the C terminus of Aβ, that may contribute to the accumulation of amyloid deposits in AD brains.

### 157P. Polymorphisms Within the Prion (PrP) and Prion-like Protein (Doppel) Genes in Alzheimer's Disease

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*Background*. The PRNP codon 129 polymorphism has been shown to influence not only phenotypic expression of prion diseases, but also cognitive impairment in the elderly. The prion-like protein gene (PRND) contains four polymorphic sites; results concerning association of PRND polymorphisms with neurodegenerative diseases have been contradictory. We present a study on the association of PRNP and PRND polymorphisms on the occurrence and age at onset of AD.

*Methods*. DNA from Polish patients with probable AD and healthy controls were studied. Coding sequences of the PRNP and PRND genes were amplified with PCR reaction. The PRND polymorphisms were analyzed by PCR product sequencing; the PRNP codon 129 polymorphism was established by restriction analysis. Results: We found a significant difference between AD and controls in PRNP codon 129 genotype distribution: in AD patients, the percentage of Val/Val and Met/Met homozygotes was higher than in the controls. A significant difference appeared also between early-onset (<70 years) and late-onset (>70 years) AD patients in the PRND genotypes. The frequencies of the Thr/Thr and Thr/Met genotypes of the PRND codon 174 were higher in late-onset than in early-onset AD patients; the PRND Thr/Thr26-Pro/Pro56-Met/Met174 genotype was over-represented among early-onset AD patients.

*Conclusions*. The PRNP codon 129 homozygous genotypes seem to be associated with the occurrence of AD. This result supports the hypothesis that the PRNP polymorphism may be of importance not only for prion diseases, but also for cognitive disorders in general. The presence of at least one allele of Thr at PRND codon 174 seems to be a risk for a later-onset, while the Thr/Thr26-Pro/Pro56-Met/Met174 PRND genotype, for an earlier onset of AD.

### 158P. NÎ-Carboxymethyllysine (CML) in Aging, Diabetes Mellitus and Alzheimer's Disease

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*Introduction*. NÎ-carboxymethyllysine (CML) is a kind of advanced glycation end-products related to oxidative stress.

*Methods*. In order to recognize the involvement of CML in Alzheimer's disease (AD) and other processes, brain tissues from Diabetes Mellitus (DM), aging and age-matched controls were immunohistochemically studied. Hippocampus, superior frontal and medium temporal gyri from patients suffering from DM without neurological disorders, patients with AD with and without DM, and age-matched controls were used for this purpose.

*Results*. CML was present in the cytoplasm of neurons in all cases, but differences in the intensity of expression, in number of possitive cells and in their topographical distribution were observed. CML was found to co-express with tau protein, showing the same neurofibrillar tangle-shape, as well as in the neuropil of neuritic plaques. A degree of CML-expression increasing from aging, to DM, to AD and to AD with DM could be observed respectively. At the same time, there was an inverse relationship between Braak and Braak's staging and the topography of CML-expression. Finally, DM cases did not show Aβ deposition and/or NFTs, however, in AD plus DM, CML-levels were higher than those from AD and DM cases.

*Conclusions*. DM should not be a risk factor for AD, but could worsen the AD evolution.

## PLATFORM PRESENTATION

### 159. Cerebellar Liponeurocytoma: Diagnosis Pitfalls

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*Background*. Cerebellar liponeurocytoma (CL) is a recently described and rare entity with a benign behaviour. Since the first description, less than 20 cases have been reported in the literature. The aim of this study was to identify cases of CL previously misdiagnosed.

*Methods*. All cases diagnosed (1994-2003) as neurocytoma, primitive neuroectodermal tumors (PNET)/medulloblastoma and cerebellar gliomas were reviewed by 2 neuropathologists. Lipomatous and neurocytic differentiations were studied on all specimens by lipid stains and immunohistochemistry.

*Results*. From a total of 36 cases, 28 were classical PNET/medulloblastoma, 6 central neurocytoma and only two CL. One of the two CL was correctly diagnosed at the initial examination and in the second case the initial diagnosis was anaplastic oligodendroglioma.

*Conclusions*. The histopathologic, immunohistochemical and clinical features of our cases of liponeurocytoma were consistent with those already described in the literature: older age of onset, peculiar histology, benign course after surgery alone.

Several of its histopathologic features may generate confusion with local PNETs , neurocytoma or glioma. Here, we show our experience on CL, emphasizing on histological criteria, differential diagnosis, and clinical behaviour of this tumor.

### 160. Malignant Transformation of Intracranial Gangliogliomas

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*Introduction*. Gangliogliomas are unfrequently occurring neoplasms of the central nervous system. They are referred as mixed neuronal and glial tumors related to their double component. Until now, the anaplastic variant as well as the malignant transformation remain unclear.

*Subjects and methods*. We report a series of 12 cases, that have been operated on between 1988 and 2003 in our hospital. Clinical and radiological data with follow-up, histological and immunohistochemical (in order to establish glial and/or neuronaldifferenciation) examinations are reviewed. Treatment choices have been surgical resection, followed or not by radiotherapy and/or chemotherapy.

*Results. i)* Histologically gangliogliomas correspond to grade I/II in the WHO classification. Nevertheless some of them show anaplastic features, indicating aggressive behavior, or anaplastic transformation secondarly. *ii)* Tumor progression was observed, obviously not only related to tumor resection, characterized by a very variable time of recurrence.

*Conclusion. i)* Gangliogliomas are not always benign tumors and prognostic factors would be useful. *ii)* One asked about the opportunity and time of a postoperative radiotherapy and/or chemotherapy.

### 161. The Case of "Angio-ganglioglioma": Diagnostic and Histogenetic Considerations of a Novel Tumor Entity—a Transitional Form Between Angioglioma and Ganglioglioma?

Liberski PP; Jesionek-Kupnicka D; Klimek A; Kordek R

We described for the first time an unusual morphology of cerebral tumor with unique clinical history, composed of three components: pilocytic astrocytoma, vasal proliferation similar to those described as arteriovanous malformations and neoplastic ganglion component. These three components were distinctively appreciated and intimately arised creating the tumor mass. Thus we propose term angioganglioglioma and we believe that it constitutes a truthful nosological entity. The relation of this entity to the historically defined angliogliomа and tumors related to ganglioglioma and dysembryoplastic neuroepithelial tumor is discussed. We thought that this lesion in regard to the clinical presentation (long course of the disease, clinical symptoms) is closely associated with gangliogliomа and, with other morphological features, also to angioglioma. Further, it may constitute a new distinct clinicopathological entity, which shares a true neoplastic and hamartomatous nature.

### 162. Study of Proliferative Markers and Tumor Suppressor Gene Proteins in Ependymomas

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*Introduction*. Ependymomas comprise about 7 to 12% of all brain tumors and are the third most common tumor in pediatric age group. The genetic pathways in ependymomas are poorly investigated unlike astrocytic tumors. This study was therefore undertaken to study various tumor suppressor gene proteins and proliferative markers in ependymomas.

*Methods*. A total of 119 cases of ependymomas of different grades were included: 17 Grade I, 54 Grade II, and 48 Grade III. Immunohistochemical staining was done for MIB-1, topoisomerase II alpha, p53 and MDM2 gene proteins by the LSAB technique.

*Results*. MIB-1 LI in grade I range from 0 to 1.47 (mean 0.81 ± 1.28), grade II from 0 to 2.47 (mean 1.59 ± 3.22) and grade III from 1 to 20.23 (mean 16.76 ± 11.93). The mean topo II alpha LI in grade I cases was 1.01 ± 1.70 (range 0-1.88), in grade II cases was 0.91 ± 0.28 (range 0-1.48) and in grade III cases was 5.53 ± 8.94 (range 0-7.15). The difference in both MIB-1 and topo II alpha LI between grade III and grade II as well as grade I was statistically significant. However, the differences in the proliferation index between grade I and II was not statistically significant. Similarly, difference in mean p53 LI of grade I cases (1.05 ± 3.11), grade II (4.44 ± 0.16) and grade III (5.50 ± 13.91) was statistically significant. MDM2 protein expression was seen in only one case of grade I and none of the grade II ependymomas; however, it was positive in 18 of 48 cases of grade III (mean 0.38 ± 1.10).

*Conclusion*. A positive correlation was noted of the proliferative indices with histological grades of ependymomas. Thus, proliferative markers were found to be higher in grade III tumors and there-

fore can be used as an adjunct to histological features for proper grading of these tumors. p53 protein expression was low in grade I and II tumors thereby indicating that p53 alterations are not involved in the pathogenesis of progression of ependymomas from lower to higher grades. This study found low incidence of MDM2 protein expression which needs further confirmation from other centres on larger number of cases.

## POSTERS

### 163P. Adult Cerebellar Neuroectodermal Tumor with Predominant Pilocytic Pattern, Focal Lipidization and Myoid Differentiation

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*Background*. Lipidization is rare in neuroepithelial neoplasms, encountered sporadically in astrocytoma, ependymoma, medulloblastoma and cerebellar liponeurocytoma. Myoid differentiation is also very infrequent in neuroglial neoplasms. We report a unique combination of these features in an adult cerebellar tumor.

*Clinical history*. A female patient aged 63 years presented with headache due to raised intracranial pressure and CT scan showed a large well-circumscribed right cerebellar mass. After tumor excision there was no recurrence in 8 years follow-up.

*Results*. Histology showed a tumor with variegated tissue pattern dominated by astrocytic cells exhibiting some biphasic appearances, suggesting pilocytic astrocytoma. Many areas revealed oligodendroglia-like cells, with palisading in places. There was also an extensive lipidization in GFAP positive tumor cells. A few scattered myoid cells were present demonstrating desmin and myoglobulin positivity. Although occasional mitotic figures, vascular proliferation in places and small ischemic lesions were noted, the tumor had generally mild pleomorphism and low proliferation index. Electron microscopy showed only lipidized glial cells and no ependymal differentiation.

*Conclusion*. This is a low-grade cerebellar neuroectodermal tumor with indolent course and long-term survival after surgical excision. The appearances are consistent with cerebellar liponeurocytoma. In addition to focal mesenchymal (lipoid and myoid) differentiation, there were predominant glial areas with features of pilocytic astrocytoma, broadening the spectrum of differentiation in this rare entity.

### 164P. Pigmented Pleomorphic Astrocytoma

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University of Bergen, Haukeland University Hospital, Norway.

*Background*. Pigmented neuroepithelial tumors are rare brain tumors. We here present an unusual case of pigmented astrocytoma.

*Clinical history*. A 19-year-old woman developed bitemporal visual field defects after a trauma to her eye. A suprasellar, well-circumscribed, partly cystic tumor, 3.2 cm in diameter was found by CT. The brown tumor was found to grow along the optic nerves. Small pigmented spots on the optic nerves, presumed to represent tumor tissue, were not resected to avoid damaging the optic nerves. The patient was therefore given radiotherapy postoperatively. She was free of relapses at last follow-up 12 years after the operation.

*Histopathology*. The tumor cells were pleomorphic and many were spindle shaped growing in fascicles. The cells had large, pleomorphic, hyperchromatic nuclei and abundant eosinophilic cytoplasm. Very few mitoses were found (less than 2 per 50 high power fields). Many tumor cells contained pigment granules. Histochemical staining techniques and electronmicroscopy confirmed that the pigment was melanin. Reticulin fibers surrounded individual cells and small groups of cells. The tumor cells, including the pigmented cells showed immunoreaktivity for GFAP, S-100 protein and vimentin. The Ki-67 labelling index has 3%. The tumor was diagnosed as a pleomorphic, possibly anaplastic, astrocytic tumor with melanin pigmentation.

*Discussion*. Only 4 cases of melanotic astrocytomas have been reported previously. One was a pilocytic astrocytoma, one was a ganglioglioma with pleomorphic xanthoastrocytoma (PXA) as the glioma component, and 2 were diagnosed as PXA.

### 165P. Histological Variation in Recurrent Supratentorial Ependymomas

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*Introduction*. Supratentorial ependymomas are not common. Their site of origin may not be clear from the radiological appearances and difficulty in diagnosis occurs at first presentation. Two such cases are described.

*Material and methods*. Routine techniques and immunohistochemical methods were used.

*Case studies and results*. The first patient presented aged 18 with a right parietal lesion, this was diagnosed as a glioma as it was GFAP positive and the tumor cells were negative for neural markers although it was cellular and mitoses were present. Radiotherapy was given. Seven years later it recurred and histologically showed typical glio-vascular pseudorosettes of an ependymal tumor. Foci of necrosis and mitotic activity were present, so a diagnosis of anaplastic ependymoma was made. Further debulking was carried out 7 years later, histologically this was also an anaplastic ependymoma with more necrosis. Chemotherapy was given but the patient died one year later. The second patient initially presented at 8 years of age and a parietal lobe tumor was removed. Histologically this had a varied appearance with clear cells but no definite pseudorosettes were seen; the tumor was GFAP positive and after referral was diagnosed as an anaplastic ependymoma. It has recurred 5 times but contains more glio-vascular pseudorosettes. Necrosis is present with focal vascular proliferation, as in an anaplastic ependymoma.

*Conclusions*. Variation of histology does occur in supratentorial ependymomas. The best treatment is by complete surgical removal.

### 166P. An Immunohistochemical and Ultrastructural Study of Malignant Ependymoma

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A 11-year-old female presented in 1990 with a recent onset of headache and visual field disturbance. Magnetic resonance imaging revealed a rt posterior carosal mass, which was well demarcated and well contrast enhancing. She underwent partial tumor resection.

Tumor lacked typical light microscopic features of ependymoma and electron microscopic study did not show the diagnostic hallmarks of ependymoma, including complex intercellular junctions, surface microvilli, cilia and microrosette formation. Postoperatively localized radiation therapy and chemotherapy was administered and she had remained well for 4 years. In 1999 she developed severe headache and CT revealed marked hydrocephalus and rt thalamic tumor which was partly cystic and partly solid. Tumor was subtotally removed and four months later CT revealed dissemination around subarachnoid space via CSF. Histological examination revealed undiferenciated ependymoma which composed of semioval cells. Tumor had mitotic figures and rosette formation. Stroma showed more marked vascular and adventitial fibroblastic proliferation. Reticulin stain shows a rich reticulin network in the fibrosarcomatous area. Electron microscopic examination revealed specific junctions of apposed cell membranes, microvilli and cilia. Above findings showed that tumor was progressively tend to malignancy.

### 167P. Papillary Ependymoma of the Pineal Region

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Primary papillary tumors of the central nervous system are rare. We have encountered a series of 6 papillary tumors of the pineal region (PTPR) with distinctive features that appear to represent a clinicopathologic entity. The tumors occurred in 4 females and 2 males, ranging in age from 19 to 53 years. Imaging studies showed a large well-circumscribed mass in the pineal region. The tumors were characterized by an epithelial-like growth pattern, in which the vessels were covered by a layer of tumoral cells. In papillary areas, the neoplastic cells were large, columnar, with a clear cytoplasm. Nuclei, round or infolded, were found generally at the basal pole of tumoral cells. Immunohistochemically, the tumor cells showed strong staining for cytokeratin, S 100 protein, neuron specific enolase, and vimentin, but only weak or no staining for epithelial membrane antigen and glial fibrillary acid protein. Ultrastructural examination of two cases revealed abundant rough endoplasmic reticulum with distended cisternae filled with secretory product, microvilli, and perinuclear intermediate filaments. The morphofunctional features of these PTPR are similar to those described for ependymal cells of the subcommissural organ and the PTPR may be derived from these specialized ependymocytes.

### 168P. Expression of Alpha-Internexin in Retinoblastoma

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Retinoblastoma is a rare malignant tumor of infancy with photoreceptor and glial differentiation. However, differentiation toward neuronal components has not been fully examined yet. We have carried out immunohistochemical neuronal markers of 5 non-familial retinoblastomas in comparison with the neuronal markers expressed in the developing normal human retina, especially for neuronal intermediate filament such as peripherin, alpha-internexin, and neurofilament. In addition, calbindin 28KD, a marker for horizontal cells was also examined. During the first trimester, there were 2 different phases of neuronal differentiation. Ganglion cells differentiated in earlier phase and co-express had alpha-internexin and peripherin from the beginning of their migration. Neuron of inner nuclear layer began to migrate later period and express alpha-internexin only. However, neurofilament was not expressed neither of these 2 types of neuroblasts during the first trimester and calbindin 28KD was not found in this period. In all cases of retinoblastoma, alpha-internexin was revealed but peripherin was disclosed in none of them. Calbindin 28KD was shown in only few of the tumor cells. These results suggest that differentiation toward the neurons, especially to the inner nuclear layer is the common phenomenon in reteinoblastoma and differentiation toward horizontal cells also seemed to be present in this tumor.

### 169P. Central Liponeurocytoma—A Case Report

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*Background*. In the 2000 WHO classification of tumors, cerebellar liponeurocytoma is defined as a rare cerebellar neoplasm with advanced neuronal and focal lipomatous differentiation. Although rarer, central neurocytoma shows similar lipomatous differentiation. We report a case of central neurocytoma with lipomatous change arising in the Monro's foramen in a 25 year-old male.

*Clinical summary*. Using imaging, the patient with right paraplegia was found to have a tumor, 3 cm in diameter, in the left lateral and third ventricles with hydrocephalus. A total resection of the tumor was performed. One year later, a second subtotal resection with postoperative irradiation was performed against the recurrent tumor.

*Results*. Histologically, the specimens from the first and second resections showed almost similar features. The tumor consisted of small, uniform cells with round nuclei and scant cytoplasm. The neuropil islands were immunopositive for synaptophysin. The characteristic finding was clusters of tumor cells with lipid droplets in their cytoplasm. MIB-1 labeling index was 2.0% in the first specimen and 3.8% in the second. Ultrastructurally, some synapse-like structures with dense core vesicles, synaptic vesicles and desmosome-like membrane thickening were shown. Some tumor cells contained intracytoplasmic lipid droplets. Conclusions. Three cases of central neurocytoma with lipomatous change have so far been reported. In a report by George and Scheithauer (2001), this tumor called "central liponeurocytoma."

## POSTERS

### 170P. Immunohistochemical Investigation into the Astrocytic Expression of GFAP Following Ethidium Bromide Injection in the Brainstem of Wistar Rats Submitted to the Diabetogenic Model of Streptozotocin

Bondan EF; Lallo MA

University Paulista (UNIP), São Paulo, Brazil.

*Background*. Diabetes mellitus (DM) is known to delay inflammatory response and tissue repair. Following ethidium bromide (EB) gliotoxic injury, central nervous system repair is invariably made at some degree with participation of surviving astrocytes. The purpose of this study was to observe if there is any interference in astrocytic response following EB injection in rats submitted to streptozotocin diabetogenic model.

*Method*. Wistar rats received a single intravenous injection of streptozotocin for DM induction (50 mg/kg) and, 10 days after, were injected with 10 microlitres of 0.1% EB (group I) in the basal cisterna of the brainstem. Non-diabetic rats were also injected with EB (Group II). All rats were perfused by the heart with 10% buffered formalin solution from 24 hours to 31 days after, collecting brainstem samples for GFAP immunohistochemical investigation (avidin-biotin method). Astrocyte density in the lesions of 31 days was compared between both groups.

*Results*. Diabetic and non-diabetic rats presented similar lesions induced by the gliotoxic agent. Astrocyte disappearance from the central area was found 24 hours post-injection and peripheral astrogliosis reaction began at 3 days with increased immunoreactivity to GFAP. No statistically significant difference was found in astrocyte density at 31 days in the EB-induced lesions from both groups.

*Conclusion*. No evidence was found that DM impaired the development of the glial scar after gliotoxic injury.

### 171P. Remyelination is Delayed after Ethidium Bromide Injection in the Brainstem of Diabetic Rats

Bondan EF; Lallo MA; Sinhorini IL

University Bandeirante of São Paulo, Brazil.

*Background*. Schwann cell disturbance followed by segmental demyelination in the peripheral nervous system occurs in diabetic patients. In the ethidium bromide (EB) demyelinating model, these cells invade and contributed to myelin repair in the central nervous system (CNS). As Schwann cell remyelination in the CNS is a well known event in this gliotoxic model, the aim of our investigation was to observe the behaviour of such cells after local EB injection in the brainstem of streptozotocin diabetic rats.

*Method*. Forty adult Wistar rats were used, from which 25 received a single intravenous injection of streptozotocin (50 mg/kg), being submitted 10 days after to a local injection of 10 microlitres of 0.1% EB (n = 15) or 0.9% saline (n = 10) solution into the cisterna pontis. Ten microlitres of EB were also injected in non-diabetic rats (n = 15). The rats were anaesthetized, perfused through the heart from 7 to 31 days after EB or saline injection and brainstem sections were collected and processed for light and transmission electron microscopy studies.

*Results*. Diabetic rats presented delayed macrophagic activity and lesser remyelination in comparison to non-diabetic rats. Although oligodendrocytes were the major remyelinating cells in the brainstem, Schwann cells still invaded EB-induced lesions, firstly appearing at 11 days in non-diabetic rats and by 15 days in diabetic rats.

*Conclusions*. Results indicate that short-term streptozotocin-induced diabetes hindered both oligodendrocyte and Schwann cell remyelination.

### 172P. Astrocyte Immunoreactivity to GFAP and Vimentin Following Ethidium Bromide Injection in the Brainstem of Wistar Rats

Bondan EF; Lallo MA; Graça DL

University Paulista (UNIP), São Paulo, Brazil.

*Background*. Ethidium bromide (EB) is known as a gliotoxic agent that causes focal astrocytic and oligodendroglial disappearance.

*Objective*. Astrocyte immunoreactivity to Glial Fibrillary Acidic Protein (GFAP) and Vimentin (VIM) was investigated after EB injection.

*Method*. Adult male Wistar rats were taken as histologic controls (group H) or injected into cisterna pontis with 0.1% EB (group E) or 0.9% saline solution (group C). Brainstem samples were collected from 24 hours to 31 days post-injection for GFAP and VIM immunohistochemical staining using avidin-biotin method.

*Results*. In group E, extensive lesions were seen in the pons and mesencephalon, with astrocyte disappearance from the central area 24 hours post-injection. Macrophagic infiltration and peripheral astrocytic reaction were noted after 3 days. Marginal astrocytes presented increased immunoreactivity to GFAP and reexpression of VIM, the last one confined to the edges of the injury site. In group C, discrete pontine lesions were observed, showing central astrocyte preservation and a peripheral GFAP staining less intense comparing to group E. No immunoreactivity to VIM was noted in such astrocytes.

*Conclusions*. Astrocytes from the edges of the EB-induced lesions presented increased immunoreactivity to GFAP and reexpression of VIM.

### 173P. Ethidium Bromide Induces Local Blood-Brain Barrier (BBB) Disruption in the Brainstem of Wistar Rats

Bondan EF; Lallo MA; Dagli MLZ; Pereira LAV; Graça DL

University Paulista (UNIP), São Paulo, Brazil; University Bandeirante of São Paulo (UNIBAN), Brazil.

*Background*. Ethidium bromide (EB) causes local astrocytic disappearance, with glia limitans disruption and supposed blood-brain barrier (BBB) breakdown The aim of this study was to investigate the BBB integrity after the injection of this gliotoxic agent.

*Method*. Adult, male, Wistar rats received 10 microlitres of 0.1% EB (group E) or 0.9% saline solution (group C) into the cisterna pontis. Brainstem fragments were collected from 24 hours to 31 days post-injection for ultrastructural study and Glial Fibrillary Acidic Protein (GFAP) immuno-histochemical staining (avidin-biotin method) for astrocyte observation. Some animals received colloidal carbon ink by intravenous route at the same periods.

*Results*. In rats from group C, there was no sign of astrocyte loss and no leakage of ink from blood vessels in the injection site. In group E, astrocyte disappearance began at 48 hours and some areas were

still devoid of astrocytic processes 31 days after. Leakage of carbon particles was seen from 48 hours to 7 days in the EB-induced lesions. Tight junctions did not show any detectable ultrastructural change due to the lack of perivascular astrocytes.

*Conclusion*. The gliotoxic agent induces reversible disruption in the BBB integrity from 2 to 7 days post-injection.

### 174P. Immunosuppression Interferes with Rat Brainstem Remyelination in the Ethidium Bromide Gliotoxic Model

Bondan EF; Lallo MA; Graça DL

University Bandeirante of São Paulo (UNIBAN), Brazil.

*Background*. Lymphocytes are present within ethidium-bromide (EB)-demyelinated lesions in the central nervous system (CNS) and the possibility of its participation in possible immune-mediated responses to the detached myelin sheaths can not be ruled out. This study aimed to investigate the consequences of lymphocytic interference with the immunosuppressive agents dexamethasone (Dx) and cyclosporine (CsA) in CNS repair after local EB injection.

*Method*. Adult Wistar rats received 10 microlitres of 0.1% EB solution into the cisterna pontis. Some were treated intraperitoneally with Dx (3mg/kg/day, group I) or Csa (10mg/kg/day, group II) during the experimental period; others were not immunosuppressed (group III). Three animals from each group were perfused with 4% glutaraldehyde at 7, 11, 15, 21, and 31 days following EB injection. Brainstem slices were collected and processed for transmission electron microscopy studies.

*Results and conclusions*. Rats from group I showed greater amounts of myelin-derived membranes than non-immunosuppressed rats, suggesting a delay in the macrophage activity of removing myelin debris. Rare lymphocytes were found. Oligodendrocyte remyelinating activity also showed a delayed pattern, with clear predominance of naked axons. Although neovascularization was decreased, astrocytic reaction was much greater. In rats from group II, the most important finding was the presence of a higher density of oligodendrocytes near remyelinating axons at the periphery. Lymphocytes were still present. Results from group II suggest that IL-2 suppression by CsA had a proliferative effect on oligodendrocyte progenitors.

### 175P. Neuropeptide Y Expression in the Rat Hippocampus Induced by Trimethyltin Intoxication

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Neuropeptide Y (NPY) is a neuromodulator that regulates blood pressure, circadian rhythms, and a variety of behavior. Trimethyltin (TMT) induces neurodegeneration in the hippocampus, piriform cortex, leading to the neurological deficits such as tremors, seizures, and hyperactivity.

*Methods*. Sprague-Dawley rats were administered a single dose of TMT (i.p. 9 mg/kg [A group] or 12 mg/kg [B group]). Brain tissues were fixed with Bouin's fixative, and embedded in paraffin for NPY immunohistochemistry. The Gallyas-Braak impregnation was conducted to detect the neurodegeneration. Expression of NPY mRNA was estimated by the real time TaqMan method.

*Results*. NPY- positive neurons were mainly observed in the CA1 pyramidal layer and dentate hilus of the hippocampus. Increased number of NPY-positive neurons in these subfields was found on day 4 after TMT injection (A group). Neurodegeneration was shown in CA1 and CA3 by day 4. In B group, numerous dentate granule cells were also immunostained on day 3. Major neurodegeneration was observed in granule cells, but few in CA1 or CA3. Increased NPY mRNA expression in the hippocampus was measured after day 3. It was reduced to the control levels by day 14.

*Conclusions*. The seizures induced by convulsive stimuli are known to increase NPY expression in the hippocampus, which modulate neuronal excitability by regulating glutamate release. Augmentation of NPY in the hippocampus induced by TMT may play a role in neuroprotective mechanism.

### 176P. Roles of 80-kDa and 100-kDa Hemopoietic Factor in the Brain

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*Purpose*. We have previously found that 2 new hemopoietic factors, such as an 80-kDa and 100-kDa factor produced by thymic myoid cells, preferentially stimulate the growth of monocytic lineage cells, including microglias. Furthermore, these factors have a potency to augment immune responses. These findings suggest that the 2 factors are also pleiotropic. We report here the production of these factors and their potential roles in the brain.

*Materials and methods*. Mouse and rat brains were stained with anti-80-kDa and anti-100-kDa antibody by immunohistochemistry. The 80-kDa and 100-kDa factors purified from brain cell cultures were used for the proliferation assay of microglias prepared from new-born rat brains.

*Results and discussion*. The 2 factors were detected by immunoblot in the conditioned media of the primary culture of rat brain cells and glial cell lines. Immunohistochemical studies revealed that neural cells also responded to the 2 antibodies. When these factors were added to the primary cultures of neural cells, MAPK activity was enhanced. Further, the 80-kDa and 100-kDa factors purified from the glial cell cultures stimulated microglia growth. These results suggest that the 2 new hemopoietic factors play important roles in brain physiology such as microglia growth and differentiation, and neural cell survival.

### 177P. Effect of Chronic Lead Exposure on Proapoptotic Bax and Antiapoptotic Bcl-2 Protein Expression in Rat Hippocampus In Vivo

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Despite reduction in environmental lead, chronic lead exposure still posess a public health hazard, particularly in children, with devastating effects on developing CNS. To investigate the mechanism of this neurotoxicity, young and adult rats were used to study whether exposure to low concentrations of lead could induce apoptosis in hippocampus. 2 to 4-week-old and 12 to 14-week-old rats received lead acetate in concentration of 500 ppm for 40 days. Control animals received deionized distilled water. In lead treated groups, the Blood lead levels was increased by 3- to 4-folds. Light and electron microscopical study of hippocampus revealed increased apoptotic cells. Western blot analysis of Bax and Bcl-2 (pro- and antiapoptotic gene products respectively) indicated high-

er expression of Bax protein and no significant change in bcl-2 expression and accordingly increased the Bax/Bcl-2 ratio compared to control group, confirming the histological study. In conclusion these data suggest that neurotoxicity of chronic lead exposure in hippocampus in vivo may partly be due to facilitation of apoptosis.

## PLATFORM PRESENTATIONS

### 178. ARA—Adult-Onset Autosomal Recessive Ataxia with Thalamic Lesions

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*Introduction*. Late-onset autosomal recessive spinocerebellar ataxia is very rare; typically well-defined recessive ataxias have an early onset. However, recently, patients with adult-onset Friedreich's ataxia have been identified by genetic testing.

*Aim*. To describe 2 unusual kindreds with adult-onset ataxia and thalamic lesions detected by brain MRI.

*Patients and methods*. Clinical, laboratory, and pathological features of 2 autopsied patients in 2 families are characterized.

*Results*. Two unusual kindreds with adult-onset ataxia have been found in Finland. In the first family 2 sisters and the brother of 5 siblings and in the second family 3 sisters of 11 siblings were affected by progressive ataxia starting at the age of 30. Clinical findings include gait ataxia, dysarthria, nystagmus, peripheral neuropathy, mild impairment in cognition and, in one patient, epileptic seizures. MRI showed symmetric thalamic lesions, changes in brainstem gray matter, and white matter changes in the cerebellum. Autopsy of 2 patients revealed neuronal degeneration with a peculiar vacuolar change in the thalamus. Neuronal and secondary tract degeneration was observed in the spinal cord, the cerebellum and the brainstem, suggesting a spinocerebellar degeneration. The disorder appears to be autosomal recessive. Genetic and sequence analysis of the frataxin gene and comprehensive examinations excluded Friedreich's ataxia.

*Conclusion*. Adult-onset recessive ataxia with bilateral thalamic lesions in 2 families seems to represent a distinct hereditary spinocerebellar ataxia.

### 179. Polyglutamine Pathology of Dentatorubral-Pallidoluysian Atrophy

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*Background*. Dentatorubral-pallidoluysian atrophy (DRPLA) is a hereditary neurodegenerative disorder caused by the expansion of a CAG repeat encoding a polyglutamine (polyQ) tract in the disease protein. In spite of the restricted degeneration of the dentatorubral and pallidoluysian systems, DRPLA patients show a variety of clinical symptoms including dementia.

*Methods*. To elucidate the prevalence of polyQ pathology, we examined immunohistochemically 4 autopsy brains of DRPLA using a monoclonal antibody 1C2 against expanded polyQ stretches.

*Results*. In contrast to the restricted lesions, labeling appeared in many neurons in a wide range of CNS regions far beyond the affected systems. The extent of polyQ pathology dramatically varied depending on the CAG-repeat sizes, showing the most striking change of frequency in the cerebral cortex. The DRPL systems and pontine nuclei were the constantly involved regions with high frequency. In neuronal nuclei, polyQ accumulated in a diffuse pattern with occasional inclusion formation. The intranuclear inclusions were co-localized with some transcriptions factors, and also interacted with nuclear bodies. In the cytoplasm, labeling was scattered as small granules, being ultrastructurally present in a subset of lysosomes. The results suggest that DRPLA may involve much wider brain regions than previously recognized, and that the variable lesion distributions depending on the CAG repeat sizes may be responsible for the considerable heterogeneity of the disease phenotypes.

### 179 bis. Unusual Presentation in a SCA17 Family

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Centro Regionale di Neurogenetica AS6 Lamezia Terme, Italy.

*Background*. SCA 17 is an autosomal dominant cerebellar ataxia caused by a CAG repeat expansion in the TATA box—binding protein gene (TBP) that typically starts with ataxia; only later patients develop behavioral symptoms.

*Methods*. We have identified a 4-generation Southern-Italian family with 16 affected patients of whom 11 were clinically followed. Western blot analysis on lymphoblasts, PCR analysis and DNA sequencing have been performed on affected, at risk and healthy individuals of the family compared to unrelated healthy and diseased controls. Neuropathology and immunohistochemistry were performed in one case.

*Results*. Behavioral symptoms and frontal impairment dominate the early stages of the disease preceding ataxia, rigidity and dystonic movements. The disease was caused by a stable 52 CAG repeat expansion of the TBP gene and the age of onset (ranging from 17-53) was not determined by the number of CAG repeat. Neuropathological examination showed cortical, subcortical and cerebellar atrophy. Purkinje cell loss and gliosis, pseudo-hypertrophic degeneration of the inferior olive, marked neuronal loss and gliosis in the caudate nucleus and in medial thalamic nuclei were salient features together with neuronal intranuclear inclusions (NIIs) stained with antiTBP and anti-polyglutamineIC2 antibodies. The characteristics of this family broaden the SCA17 clinical picture.

### 179 tris. Termination of Expression of Mutant Ataxin-1 Results in Arrest and Partial Reversal of Purkinje Cell Pathology in Spinocerebellar Ataxia Type 1 (SCA1)-Transgenic Mice

Zu T; Orr HT; Clark HB

University of Minnesota.

*Background*. A previously described transgenic model (line-B05) of SCA1 demonstrated that Purkinje cells (PC) exhibit cytoplasmic vacuolation and dendritic dwindling and that behavioral ataxia precedes PC death. The current study was performed to determine the effects of stopping production of transgenic mutant ataxin-1 on the clinicopathological phenotype in SCA1 transgenic mice.

*Methods*. Double transgenic mice were generated which express a tetracycline-regulated transactivator (tTA) driven by the PC-specific promoter, Pcp2(L7); and an SCA1 gene with 82 CAG repeats driven by a tetracycline responsive element (TRE). The mutant-SCA1 gene is expressed in the absence of doxycycline (dox) and not expressed in its presence.

*Results*. Double-transgenic mice homozygous for TRE expressed mutant ataxin-1 mRNA, in the absence of dox, at levels

equivalent to B05. These mice had impaired rotarod performances and PC pathology similar to age-matched B05 mice. Treatment with dox between ages 6 to 12 weeks resulted in improved PC morphology when compared to untreated TRE mice both at 6 and 12 weeks. Ataxia measured by cage behavior and rotarod also improved. Dox treatment from ages 12 to 16 weeks resulted in pathological improvement over 12 and 16 week untreated mice.

*Conclusion*. PC structure and function can be improved in a model of SCA1 if production of mutant ataxin-1 is halted. The temporal limits of this phenomenon currently are being investigated.

## POSTERS

### 180P. Clinicopathology of SCA1 in Non-Endemic District—2 Autopsy Cases

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The families of SCA1 in Japan are reported to originate exclusively from Tohoku District, Miyagi-Yamagata boundary (M-Y area). Two patients in 2 families diagnosed on genetic analysis are from the pedigree in Akita Prefecture, not related to the pedigrees in M-Y area. We compare these 2 cases clinicopathologically to that of the cases from M-Y area. Case 1 is a 52-year-old male patient who developed ataxia at the age of 20 and case 2 is a 27-year-old female patient with ataxia from early teens. The one originates from Yuri district and the other from Tazawa Lake district, not related to each other. The CAG repeat numbers are 48 and 99.

*Neuropathology*. Brain weight was 1100 g and 985 g. Marked atrophy of the brain stem and cerebellum. Extensive neuronal loss and gliosis was seen in the olivopontocerebellar system. The dentate nuclei, red nuclei and substantia nigra were affected. Neuronal loss and gliosis were seen in the lateral geniculate body, thalamic nuclei, and oculomotor nuclei. Much more severe and extensive involvement in Case 2 than in all cases in the prevalence district suggests that precise haplotype analysis and clinicopathological comparison of 2 different pedigrees is mandatory.

### 181P. Report of 3 Autopsy Cases of Spinocerebellar Ataxia Type 3 (SCA3) in One Pedigree

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Three autopsy cases of spinocerebellar ataxia type 3 in one pedigree were neuropathologically investigated using ordinary stained paraffin sections and immunohistochemically stained ones. Case 1 was a 59-year-old woman (duration of illness, ca. 16 years), who was also mother of cases 2 and 3. Case 2 was a 40-year-old man (duration of illness, ca. 20 years), and case 3 was a 60-year-old man (duration of illness, ca. 21 years). Genetic investigation in case 3 revealed excessive CAG repeat, followed by definite diagnosis of SCA3. There were observed similar neuropathogical findings in all 3 autopsy brains showing degeneration and/or loss of nerve cells and/or nerve fibers in cerebellar system, extrapyramidal system, ocular motor system, spinal cord, and cerebral cortex, associated with deposition of polyglutamine in some residual nerve cells immunohistochemically, which were thought compatible with SCA3.

### 183P. Attenuated Shrinkage of Pontine Neurons with Nuclear Aggregates in SCA1, SCA2, MJD, and DRPLA

Uchihara T; Iwabuchi K; Nagaoka U; Funata N; Konno H; Tobita M; Kato T; Yagishita S

Nuclear aggregates (NAs) and neurodeneration in brains from patients with CAG repeat disorders are both triggered by pathological expansion of CAG/polyglutamine repeat in corresponding gene/protein, while it remains to be clarified whether NA formation is associated with accelerated neurodegeneration or not. In an attempt to clarify a possible influence of NAs on neurons in human brains, we quantified the size and deformity of neuronal nuclei (those with or without NAs, separately) on pontine sections of autopsied brains from patients with SCA1, SCA2, MJD or DRPLA and 5 controls. Nuclear shrinkage and deformity were more marked in brains with any of these disorders than in controls, while these changes were attenuated in neurons harboring NAs. NAs of CAG/repeat disorders are presumably linked to a mechanism, which attenuates rather than accelerates nuclear shrinkage and deformity. This in vivo finding may provide a clue to constructing a rational therapeutic strategy aimed at combating against neurodegeneration associated with NAs.

### 184P. Immunohistochemical Study of Expressions of Tyrosine Hydroxylase and its Phosphorylated Form in the Cerebellum of Ataxic Mutant Mice

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Rolling mouse Nagoya (RMN) and dilute-lethal mice (DL) are hereditary ataxic mutants carrying different mutant alleles, the tottering locus on chromosome 8 and the dilute locus on chromosome 9, respectively. Expressions of tyrosine hydroxylase and its phosphorylated form at $Ser^{40}$ (phospho-TH) were examined immunohistochemically in the cerebellum of these 2 mutants. TH immunostaining abnormally appeared in some Purkinje cells in RMN and DL, but in a few Purkinje cells of littermate controls for both mutants. TH immunoreactive Purknje cells were organized into parasagittal bands in all cerebellar lobule of RMN and in the vestibulocerebellum of DL. However, no phospho-TH immunoreactive Purkinje cells were found in the cerebellum in all groups of mice, although the subsets of Purkinje cells were TH immunoreactive in the adjacent sections. The results suggest that TH expression in the Purkinje cells of the ataxic mutants abnormally increases without phosphorylation of this enzyme. As phosphorylation of TH at $Ser^{40}$ drastically accelerates its enzymatic activity, TH in the Purkinje cells seems not to be related to catecholamine synthesis.

### Using Developmental Neurobiology and Neuropathology to Refine Concepts of Developmental Disorders

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Development of the nervous system is a complex process that requires the orchestration of multiple cellular and molecular interactions at precise times during development. One essential component of CNS development is cell migration. Advances in our understanding of cell migration have significantly expanded our understanding of multiple developmental disorders of the nervous system. The best-characterized pathway of cell migration is radial, guided by the radial glial cell. Studies performed over the last four decades have characterized many of the molecular and cellular components of this pathway. Furthermore, defects in this pathway have been show to result in human malformations such as periventricular heterotopia, subcortical band heterotopia, and lissencephaly. More recently a second pathway of cell migration has been identified that is perpendicular to the radial pathway of migration. Mounting evidence indicates that this non-radial cell migration pathway is not only instrumental to normal CNS development but is likely to be the major pathway by which inhibitory interneurons arrive in the cerebral cortex. Finally, evidence now exists that non-radial cell migration also results in specific human malformations. In this presentation, I will review the cell and molecular biology underlying distinct modes of cell migration in the developing nervous system and correlate this to the development of human malformations.

### Mechanisms of Cell Damage and Death After Brain Hypoxia/Ischemia in the Neonate

Gressens P

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The pathophysiology of perinatal brain lesions seems to be complex and multifactorial, involving not only hypoxic-ischemic insult but also other pre or perinatal factors including chorioamnionitis and excess production of inflammatory mediators, hormone and growth factor deficiencies, oxidative stress and genetic factors. The development and characterization of separate and complementary animal models should permit to tease out the cellular and molecular mechanisms underlying perinatal brain lesions and accompanying neural cell death. In pre-term neonates, white matter oligodendrocyte precursors seem particularly vulnerable to insults. Different glutamate receptors, reactive oxygen species, pro-inflammatory cytokines and activated macrophages-microglia could play a key role in white matter cell death and axonal damage. At term, perinatal asphyxia leads mainly to neuronal damage in the cortex and basal ganglia. Animal models have shown that *i)* there is an early and a delayed phase of neuronal cell death; *ii)* the delayed phase can be protracted; and *iii)* there is some continuum between apoptosis (caspase-mediated pathway) and necrosis, mitochondrial metabolism being an important factor in determining the type of cell death. Several growth factors or energy substrates are able to limit the intensity of the delayed neuronal cell death. However, the effects on neuronal cell death of some growth factors such as BDNF are dependant upon the stage of brain maturation.

### Mechanisms and Management of Intracerebral Hemorrhage

Kase C

Intracerebral hemorrhage (ICH) is predominantly due to hypertension. However, a number of non-hypertensive mechanisms account for a substantial proportion of the cases. Those due to therapeutic interventions (ie, iatrogenic ICHs) constitute an important group, as they should be largely preventable.

ICH is the most serious complication of oral anticoagulant treatment. Its high mortality is related to larger hematoma size in comparison with hypertensive ICH. Some of its established risk factors are advanced age, preceding ischemic stroke, and excessively prolonged International Normalized Ratio (INR). Other more recently recognized risk factors are the presence of cerebral amyloid angiopathy and leukoaraiosis. Particular problems posed by anticoagulant-related ICH are measures for prevention, and timing of re-institution of oral anticoagulation after an episode of ICH.

ICH after thrombolytic treatment has become a common occurrence with the increased use of this therapy for acute ischemic stroke. Both intravenous (IV) and intra-arterial (IA) use of tissue plasminogen activator (tPA) are complicated by ICH, which is often fatal. Risk factors for ICH after IV tPA are primarily related to the presenting ischemic stroke, and include stroke severity (measured as the baseline National Institutes of Health Stroke Scale [NIHSS] score), and size of infarction on the baseline CT scan. For the IA use of thrombolysis, only hyperglycemia at baseline has been identified as a potential predictor of increased risk of ICH. Further search for risk factors for ICH and careful selection of candidates for IV or IA thrombolysis are likely to reduce the frequency of this complication of thrombolysis.

### The Causes of Human Cancer: A Global View

Kleihues P

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In the year 2000, 5.3 million men and 4.7 million women developed a malignant tumor, and altogether 6.2 million died from the disease. In developed countries, the overall cancer mortality is more than twice as high as in developing countries. The main reasons for the greater cancer burden of affluent societies are the earlier onset of the tobacco epidemic, the earlier exposure to occupational carcinogens, and the Western nutrition and lifestyle. In developing countries, up to 25% of malignancies are caused by infectious agents versus 8% of all malignancies in developed countries.

Tobacco consumption remains the most important avoidable cancer risk. Half of regular smokers are killed by the habit and one in 4 will die prematurely during middle age. In industrialized countries, approximately one third of malignant tumors are due to the Western lifestyle, characterized by a high caloric diet, combined with low physical activity. Tumors associated with the Western lifestyle include cancers of the breast, prostate, colon/rectum, and endometrial carcinoma of the uterus, which are much less frequent in poor countries. For some cancer types, including non-Hodgkin lymphoma, testicular cancer and brain tumors, the causative factors are still largely unknown.

Given the current trend of tobacco abuse and unhealthy lifestyle choices in many world regions, a reduction in mortality will largely depend on progress in early detection and treatment.

### Sarcomeric Proteins and Their Diseases

Laing NG

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We now know that many sarcomeric proteins are mutated in various human skeletal muscle diseases. The mutated proteins include: actin, alpha B crystallin, desmin, myosin, myotilin, nebulin, plectin, telethonin, titin, tropomyosin, and troponin. The diseases caused vary from arthrogryposis, through congenital myopathies to distal myopathies and muscular dystrophies. Broad rules about the sarcomeric protein mutations are emerging, although these are not absolute. Missense mutations tend to cause dominant disease, whereas null mutations, both nonsense and frameshift, are recessive. It has also become apparent that the protein interactome of at least some sarcomeric proteins presages the disease phenotype (eg, mutations in the thin filament proteins actin, nebulin, tropomyosin and troponin cause nemaline myopathy, mutations in desmin and alpha b crystallin cause desminopathy). Mutations in titin and beta cardiac myosin both cause distal myopathy. Does this similar phenotype arise through corruption of the normal interaction between myosin and titin? Additionally, the myosin and titin distal myopathies demonstrate rimmed vacuoles, while mutations in myosin IIa causes dominant inclusion body myopathy, whose pathology also includes rimmed vacuoles. Does this mean that other known protein interactions can productively define further candidate genes? The fact that mutations in beta cardiac myosin and titin can give rise to skeletal muscle disease without a cardiac phenotype and preferentially affect a restricted group of muscles indicates that we still have a great deal to learn about the normal biology of muscle. Devising successful treatments for sarcomeric protein diseases may challenge us for decades.

### Molecular Dissection of the Multi-protein, High Molecular Weight, Membrane-bound Presenilin Complexes

St George-Hyslop P; Gu Y; Chen F; Hasegawa H; Fraser P

The presenilin proteins (PS1 and PS2) are homologous, polytopic, transmembrane proteins that are necessary for the endoproteolytic cleavage of several Type 1 transmembrane proteins, including the amyloid precursor protein (APP). The cleavage of APP generates Abeta and a series of C-terminal stubs (epsilon-stubs). While PS1 and PS2 form independent, high molecular weight complexes, these complexes contain several components in common. The other components of the PS1 and PS2 complexes include nicastrin (a Type 1 transmembrane glycoprotein), APH-1 (a polytopic transmembrane protein with no significant homology to other proteins) and PEN-2 (a unique peptide with two transmembrane domains). Glycosylation and trafficking of nicastrin to the cell surface is necessary for the biological activity of the presenilin complexes, and nicastrin preferentially binds to the mature presenilin components. Conversely, absence of PS1 or PS2 destabilizes nicastrin. APH-1 appears to be a stable component predominantly located in the ER and may represent the initial scaffolding molecule. APH-1 binds to both mature and immature species of nicastrin and presenilin. The role of PEN-2 is less clear. Analysis of the functions of the presenilin complex components may provide clues to a novel form of endoproteolytic cleavage (regulated intramembranous proteolysis) as well as to potential targets for therapeutics for Alzheimer's disease.

## WORKSHOPS

### Mechanisms of Soluble Amyloid Aβ Accumulation

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*Background*. The accumulation of soluble Aβ oligomers is the major pathologic event of Alzheimer's disease (AD). Methods. Type, amount, and binding of the soluble form of Aβ (sAβ), that reflects the toxic, small Aβ aggregates, were analyzed in cerebral cortex from various pathological conditions.

*Results*. Soluble Aβ is composed by 3 major peptides, corresponding to the full-length 1-42/40, and 2 N-terminal truncated species, 3-42 and 11-42, with pyroglutamate at positions 3 and 11. In sporadic AD Aβ py3-42 is the dominant species, accounting for 50% of sAβ. In normal brain from young subjects sAβ is undetectable in a free state, indicating that physiological mechanisms dismiss the normally produced Aβ. ApoE plays a role in the physiological sAβ clearance, since in normal condition it forms an insoluble complex with sAβ, facilitating sAβ degradation by proteases. In subjects with Down's syndrome sAβ is already present in fetal brain, and its concentration proportionally increases with age. In cases with familial AD bearing mutations of PS1 gene there is a significant 40% increase of the N-terminal truncated sAβ species in comparison to sporadic AD. An opposite sAβ pattern, consisting in the prevalence of the full-length 1-42/40 form, is present in cognitively normal elderly subjects, confirming that the composition of sAβ drives the neurotoxicity of the molecule. In FTDP-17 cases, with mutations of Tau gene, a slight but significant increase of sAβ occurs, suggesting that a primary tau pathology may foster Aβ accumulation.

### Role of Cholesterol in Basic Biology and Treatment of Alzheimer's Disease (AD)

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Several lines of evidence suggest a role of cholesterol for in AD. First, cholesterol transport and metabolism and AD are genetically linked. The apoE4 allele is associated with higher cholesterol levels and has been shown to increase the risk of developing the disease three- to sevenfold. Second, experimental suppression of cholesterol de novo synthesis by statins strongly reduces the formation of the short and long forms of Abeta (Aβ40 and Aβ42) in vivo and in vitro. Third, epidemiological studies showed that statin treatment reduced the relative risk of AD 3-fold. Fourth, a placebo-controlled, double-blind study revealed a slower progression in AD patients that received statins (Simons et al, *Arch Neurol* 52: 346-350).

Experiments in cell culture suggest that the processing of APP by beta- and gamma- secretase is affected when cholesterol levels are lowered. Because cholesterol is required to transport APP, BACE and presenilin to compartments in which beta- and gamma-cleavage does occur, intracellular cholesterol transport regulates the Abeta-generating, amyloidogenic processing of APP. Alterations in cholesterol transport have important consequences for both, APP-processing by beta-secretase and the localization of the presenilins. Exposure of neuronal cells to cholesterol-transport inhibiting agents resulted in a marked decrease in beta-secretase cleavage products of full-length APP. Our results suggest that amount and subcellular distribution of cholesterol may be an important factor in how cholesterol alters Abeta production and the risk of AD.

### Alzheimer's Disease: Clues from Complex Genetics

Tanzi RE

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Alzheimer's disease (AD) is an age-related neurodegenerative disease with complex inheritance. While rare mutations in 3 genes are causative for the early-onset form of AD, a common polymorphism in the gene encoding apolipoprotein E (*APOE*) is a risk factor for late-onset (>60) AD. AD-linked alterations in all four genes increase the accumulation of β-amyloid in the brain. These findings have prompted recent clinical trials aimed at curbing the production of, or enhancing the clearance of Aβ. Recent studies have indicated the existence of additional AD genes. To localize these genes, we have completed analysis of a high-resolution genome screen of the full National Institute of Mental Health (NIMH) family sample. We performed a 9 centimorgan genome screen of 437 families with AD - the highest resolution whole genome screen of the largest uniformly ascertained and evaluated AD sample performed. We observed a "highly significant" linkage peak on chromosome 19q13 consistent with *APOE*. We also observed additional locations for putative AD genes on several chromosomes meeting criteria for "suggestive" linkage. The linkage peaks on chromosomes 9 and 10 are particularly compelling and have been intensively followed up. Data will be presented for promising positional candidate genes on these chromosomes including the *APBA1* (encoding X11) and *IDE* (encoding insulin degrading enzyme) genes on chromosomes 9 and 10, respectively. Ultimately, the identification of novel AD genes should allow for improved prognosis and diagnosis of AD, as well as the development of new therapeutic strategies for the treatment and prevention of this disease.

### Alzheimer's disease: Modulation of the APP/Aβ Pathways Towards Rational Therapeutic Intervention

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As the molecular basis of Alzheimer's disease becomes more clearly defined, then more therapeutic targets can be validated and taken into the clinic. Currently there are several approaches to the Aβ amyloidogenic pathway which have now reached early phase clinical trials:

*1)* γ-secretase inhibition. The search for effective modulators of the proteolytic processing of APP has yielded at least 7 classes of compounds. Three of these classes have now entered clinical trials, one of which has been abandoned because of adverse events.

*2)* Metal-Protein Attenuating Compounds (MPACs). Interactions of metal ions [Zn(II), Cu(II)] with Aβ have multiple effects including promoting aggregation, lipid penetration and engendering harmful redox activity. Compounds which have the property of attenuating Aβ-metal interactions are therefore of interest in promoting Aβ clearance from the brain and ameliorating Aβ toxicity.

*3)* Immunization. Immunization with Aβ promotes its clearance from the brain. This strategy, applied to humans, has caused an anticipated adverse autoimmune response. Novel epitopes on Aβ may yet provide a strategy to bypass this serious adverse effect.

*4)* Cholesterol metabolism. Lowering cerebral cholesterol may decrease Aβ production, possibly through the β-secretase pathway. The use of statins are now being actively investigated in prospective studies.

*5)* Aβ-binding proteins. Various classes of molecules have been shown to bind to Aβ aggregates in vivo, and some of these (glycosaminoglycans, for example) may inhibit fibrillization.

An increasing number of therapeutic targets within the central Aβ amyloidogenic pathway have emerged. Each in their own way may provide a definitive test of the Aβ amyloid hypothesis of Alzheimer's disease if they can be translated into the clinic.

## PLATFORM PRESENTATIONS

### 185. Soluble Aβ Shows a Different Composition in Normal Aging and Alzheimer's Disease

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[3] Sanders-Brown Center on Aging, Lexington, Ky.

*Background*. Cerebral water-soluble β-amyloid (wsAβ), that corresponds to the metastable and neurotoxic Aβ-oligomers, is composed by species variable at the N- and C-termini. The N-terminal truncated species prevail on the full-length form in brains of familial Alzheimer's disease (AD) cases, in comparison to sporadic AD. Thereby, the pattern of wsAβ species in normal aging, in which extensive Aβ load without neuronal changes occurs, may differ from that of AD.

*Methods*. We analysed the composition of wsAβ in cerebral cortex from 15 cognitively normal elderly subjects and 15 cases of AD by immunoprecipitation and immunoblotting. Then, in the 2 groups we quantified in adjacent cortical sections the Aβ plaques by immunocytochemistry.

*Results*. Cerebral wsAβ is prevalently composed by the full-length Aβ form (50%) in normal aging, and by the N-terminal truncated species 3-42 (48%) in AD. A similar pattern was detected by immunocytochemistry in the 2 groups of cases. Mass spectrometry analysis is in progress to confirm the immunochemical results.

*Conclusion*. The pattern of wsAβ peptides may account for the different effect exerted by Aβ in normal aging and AD. Then, our results suggest that the composition of Aβ may dictate the conformation of the soluble aggregates, consequently driving the toxicity on neuronal membrane.

### 186. Neuropathology of Human Alzheimer's Disease Following Immunization with Amyloid β-peptide

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*Background*. Amyloid β-peptide (Aβ) plays a key role in the pathogenesis of Alzheimer's disease (AD). Immunization with Aβ in a transgenic mouse model of AD reduces both age-related accumulation of Aβ in the brain and associated cognitive impairment.

*Methods*. The first human neuropathology following immunization with Aβ (AN-1792) was compared with unimmunized cases of AD (n=7).

*Results*. The following unusual features were present in the immunized case: *i)* extensive areas of neocortex contain very few Aβ plaques, despite diagnostic neuropathological features of AD; *ii)* areas of cortex devoid of Aβ plaques contain densities of tangles, neuropil threads and cerebral amyloid angiopathy similar to unimmunized AD but lack plaque-associated dystrophic neurites and astrocyte clusters; *iii)* in some regions devoid of plaques Aβ immunoreactivity is associated with microglia; *iv)* a T lymphocyte meningoencephalitis; and *v)* infiltration of cerebral white matter by macrophages.

*Conclusions*. Findings *i* to *iii* strongly resemble the changes seen after Aβ immunotherapy in mouse models of AD and suggest the possibility that the immune response generated against the peptide elicited clearance of Aβ plaques in this patient. The T lymphocyte meningoencephalitis is likely to correspond to the side effect seen in some other patients who received AN-1792.

### 187. Vascular Dementia, Alzheimer's Disease and Mixed Dementia: the Weight of Various Mechanisms

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The relative importance of vascular and Alzheimer lesions, their interaction in the development of cognitive impairment and the very existence of mixed dementia induced by potentiation of both mechanisms remain controversial. The role of strategic areas of the brain involved in the cognitive decline induced by vascular lesions remains also poorly understood. We performed a prospective clinicopathological study in elderly patients of a long-stay care unit. The severity of dementia was assessed by psychometry less than 6 months before death, a volumetric study of Mesulam's functional zones affected by vascular lesions was made and the density of neuritic plaques, Aβ focal deposits, and neurofibrillary tangles in the temporal and frontal isocortex was quantified. The severity of cognitive impairment was significantly correlated with the total volume of infarcts, and in a multivariate model the volume destroyed in the limbic and heteromodal association areas, including the frontal cortex and the white matter explained 50% of the variability in cognitive decline. The density of Alzheimer lesions was significantly lower when vascular lesions were present. This study confirms that infarcts located in strategic areas have a role in cognitive impairment. It provides support for the validity of the mixed dementia concept and suggests that the pathological processes involved in Alzheimer's disease and multiple infarct dementia are synergistic and cumulative.

### 188. A Quantitative Study of Regional Accentuation of Neurofibrillary Degeneration in Familial and Non-familial Alzheimer's Disease

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To investigate regional variation of neurofibrillary degeneration in familial Alzheimer's disease (AD) and non-familial AD, we quantified the density of intracellular neurofibrillary tangles (i-NFT), extracellular NFT (e-NFT) and NFT-free nerons in the hippocampal cortex which was divided into 5 subdivisions: the sectors of cornu ammonis (CA) 4, CA3, CA2 and CA1 and the subiculum. Six cases of familial AD with amyloid β precursor protein (APP) 717 gene mutation and 7 with presenilin (PS)-1 gene mutation, 33 cases of apolipoprotein E4 (E4) allele-verified non-familial AD and 6 control cases were studied. The APP717 mutation cases showed more severe neuronal loss and much higher e-NFT densi-

ty than the non-familial AD cases in sectors CA2 and CA1. The PS-1 mutation cases showed more severe neuronal loss and abundant e-NFT formation than the non-familial AD cases in the CA sectors, but i-NFT density was not significantly different between these groups. The E4 allele frequency in the non-familial AD cases paralleled the e-NFT density in sector CA1 and the subiculum. APP717 and PS-1 mutation were thought to modify development of NFT formation in sectors CA2 and CA1. As NFT development in sector CA2 had no correlation with disease duration or E4 allele frequency in non-familial AD, it may occur only under a limited and specific condition related to APP717 and PS-1 mutation. In addition, PS-1 mutation may accelerate the degenerative process from a surviving neuron with NFT into neuronal death via an NFT-dependent pathway.

### 189. Comparative Study of Neurofibrillary Tangles in Alzheimer's Disease and Down Syndrome by Transmission (TEM) and Scanning (SEM) Electron Microscopy

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[2] Department of Neurology, National Sagamihara Hospital, Sagamihara, Japan.

*Background*. To elucidate the fine structures of neurofibrillary tangles, several methods and techniques, such as negative-stained electron micrography, high-resolution electron microscopy with image analysis, X-ray diffraction, freeze-etching with metal replication and atomic force microscopy, have been performed, leading various three-dimensional models of paired helical filaments (pHs).

*Materials and methods*. We examined the brains with Alzheimer's disease and Down syndrome by TEM and SEM. For SEM, small fragments ($3 \times 3 \times 5$mm cubes of Ammon's horn in each case were treated with a routine manner, and macerated with 0.1% osmium tetroxide for 72 hours using a modified Tanaka and Mitsushima's methods. The samples were observed in a Hitachi S-4500 SEM at 10-kV and 7-mm working distance.

*Result*. By TEM, straight filaments measured about 15 to 25 nm in diameter. As to the paired helical filaments (PHFs) were up to 33 nm and constricted at 75- to 80-nm interval, the other was 16 to 18 nm and at 35 to 40 interval. By SEM, 2 profiles of PHFs were observed: one; 34 nm and 80 nm interval, other: 17 nm and 40 nm interval.

*Conclusion*. The fine structures of the tangles in both diseases were virtually identical but different in frequency, being much more predominant of PHFs of short intervals in the brains with Down syndrome.

### 190. Effect of the ϵ2 and ϵ4 Alleles in the Amyloidogenesis

Gironès X; Guimerà A; Cruz-Sánchez C-Z; Ortega A; Cruz-Sánchez FF

*Introduction*. Apolipoprotein E (ApoE) is a glycoprotein which has an essential role in the metabolism of lipoproteins. The ϵ2 allele of ApoE has been related to the hypertrigliceridemia. On the contrary, ϵ4 allele has been related to amyloid angioathy (AA) and Alzheimer's disease (AD).

*Methods*. The frequency of the ApoE genotype was correlated with the neuropathological changes of AD. A representative region from neocortex from 41 AD cases and 75 "potential" controls cases were studied using classical histological techniques and immunohistochemistry for tau protein and amyloid-β.

*Results*. The frequency of the ϵ4 allele was significantly increased not only in AD patients, 13 of 75 potential control cases which exhibited histological changes associated with AD (Braak & Braak II and III), were found to be ϵ4 positive. The significant differences found in the distribution of ApoE allele frequencies were more marked when these cases were excluded from the control group and included in the AD group as "pre-clinical" forms. Fourteen cases from the potential control group show neurofibrillary tangles in hippocampus and cerebral cortex in absence of senile plaques or AA, all this cases show the ϵ2 allele. Conclusions: These results point to ϵ2 as an inhibitor of amiloidogenesis and related to abnormal phosphorylation of tau.

### 190 bis. The Cerebellar Cortex in Frontal Dementia: a Golgi and Electron Microscope Study

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Neuropathological alterations in frontal dementia have been extensively described in the frontal and temporal isocortex as well as in the hippocampus, consisting mainly of neuronal loss, vacuolation of the upper cortical layers and extensive gliosis of the cortex and the hippocampus. Visible swollen chromatolytic neurons in the cortex of the brain hemispheres, as well as Pick's bodies have been considered as hallmarks of the frontal dementias at the level of light end electron microscopy.

In the present study we tried to figure out the morphological alterations of the synapses in the cerebellar cortex in 2 cases of frontal dementias studied in Golgi and electron microscopy, since it is known that the cerebellum is one of the less affected structures of the central nervous system in Alzheimer's disease and frontal dementias and any change in the morphology of the synapses would emphasize therefore the importance of the synaptic factor in the pathogenesis of the dementias.

In Golgi staining an extensive loss of mossy fibres in the cortex of the vermis and the cerebellar hemispheres was seen, associated with loss of granule and Purkinje cells and marked proliferation of Bergmann's glia. In electron microscopy a tremendous loss of Purkinje cells dendritic spines were noticed associated with a wide dilatation of the presynaptic terminals of the parallel fibres that included a limited number of polymorphic synaptic vesicles. The cerebellar interneurons, namely the stellate and basket cells, were decreased in number and showed few synaptic contacts with the soma and the dendrites of Purkinje cells. The climbing fibres did not show any morphological alteration in correlation with normal controls of the same age with the patients. Large number of Purkinje cells contained osmiophilic material in the soma and the main dendritic shaft and showed an impressive loss of their axonic collaterals. Pick's bodies or Hirano bodies were nor seen either in the Purkinje cells, or in the granule cells and the interneurons of the cerebellar cortex.

The morphological changes of the synapses and the neuronal loss of cerebellar cortex enlarge the spectrum of the neuropathological alterations in frontal dementias and reveals that the widespread synaptic changes are a substantial component of the pathology of dementias.

## POSTERS

### 191P. Levels of Cerebrospinal Fluid Prostaglandin E2 are Mildly Elevated in Early Alzheimer's Disease but Decline with Increasing Severity of Dementia

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Epidemiological studies indicate that the long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) is associated with a reduced risk of developing Alzheimer's disease (AD). As the main target of NSAIDs is cyclooxygenase (COX), it has been proposed that NSAIDs protect neurones from inflammatory-mediated damage by preventing prostaglandin (PG) synthesis. To investigate further COX activity in AD, we utilized the longitudinal OPTIMA study and measured CSF PGE2 levels in 35 controls and 33 AD patients, using samples obtained on at least 3 annual visits. Cognitive status was assessed using the CAMCOG test battery. Median CSF PGE2 levels of patients and controls at initial visit did not differ significantly. However, when PGE2 levels were related to learning subscale scores there was a curvilinear dependence of CSF PGE2 on dementia severity. Early in the disease PGE2 levels rose but as episodic memory deteriorated, levels fell. In addition, the median survival time of patients with high PGE2 levels was 5 years longer than those with low levels. Our finding suggest that inflammatory mechanisms are probably more important in early phases of disease and are consistent with recent experimental evidence supporting beneficial effects of NSAIDs that do not involve COX inhibition.

### 192P. Amyloid Peptides Aggregation is Enhanced by Inflammation-related Proteins C1q and SAP

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*Background*. Alzheimer's disease and prion disease are characterized by extracellular deposition of Aβ and PrP amyloid fibrils, respectively. In both disorders, amyloid deposits are decorated by antibodies against so-called amyloid associated proteins including Serum Amyloid P component (SAP) and complement factor C1q, and are closely associated with a locally-induced, chronic inflammatory response.

*Methods*. To assess the role of C1q SAP in amyloidogenesis, we examined the effects of these molecules on the fibrillogenic properties of $A\beta_{1-42}$ and PrP106-126 synthetic peptides. Aggregation was evaluated at different times by light microscopy following Congo red or electron microscopy after negative staining.

*Results*. $A\beta_{1-42}$ generated long, straight or occasionally twisted fibrils, while PrP106-126 formed shorter, straight, unbranched fibrils. The addition of C1q and SAP to peptides suspensions resulted in a striking increase in fibril density even after one hour incubation, with generation of a large number of densely packed aggregates. Furthermore, the fibrillary structures differed from those formed by peptides alone in that they were shorter, less regular and more heterogeneous in size.

*Conclusions*. The enhancement of aggregation and the modification in fibril morphology induced by C1q and SAP are consistent with the view that these inflammation-related proteins features in fibrillogenesis, both in Alzheimer's and in prion diseases.

### 193P. Scavenger Receptor CD36 Overexpression in Brains of Alzheimer's Patients and Healthy Donors with Amyloid Plaques

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Scavenger receptors recently have been related to Alzheimer's disease, although it is still unclear whether they contribute to the pathogenesis of the disease or reflect an inflammatory response to the deposition of amyloid β-protein (Aβ). In this study we demonstrate that CD36, a class B scavenger receptor, is highly expressed in cerebral cortex of Alzheimer's disease patients and cognitively normal aged subjects with diffuse amyloid plaques compared with age-matched amyloid-free control brains. Moreover, in vitro experiments indicated that Aβ is able to induce CD36 expression in neuronal cells after 24 hours treatment. The interaction between CD36 and Aβ has been reported to trigger oxidant production by macrophages and microglia. In line with this observation, we found an increased presence of oxidative markers in brains showing Aβ loads and CD36 overexpression, independently of the occurrence of Alzheimer's disease pathologic features. Therefore, the link between Aβ load, CD36 expression and release of oxidants is confirmed in this study on human brains and cultured cells, but the conclusion that these events are necessarily associated with the development of AD is not supported by the current data.

### 194P. Neuroinflammation in Alzheimer's Disease, Frontotemporal Dementia and Dementia with Lewy Bodies

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*Introduction*. Insoluble protein deposits characterise a number of neurodegenerative disorders. The A-beta protein is thought to stimulate inflammation in Alzheimer's disease (AD) and inflammatory events are thought to play an important role in neurodegeneration. We investigated the association between inflammation and neurotoxicity in numerous neurodegenerative disorders.

*Methods*. Activated microglia, plaques, tangles, Pick bodies and neuronal loss were analysed in cortical tissue from 8 sporadic and 6 presenilin-1 (PS-1) AD, 8 dementia with Lewy bodies (DLB), 5 frontotemporal dementia (FTLD), 8 Pick's disease (PiD) and 10 control cases.

*Results*. A-beta pathology was similar in AD and DLB cases but was not a feature of control, FTLD and PiD cases. Only PiD and AD cases had significant tau pathology. Activated microglia and neuronal loss were seen in AD, PiD and FTLD but not controls or DLB. Activated microglia were only associated with pathology in AD although tau-positive microglia were found in FTLD. In PS-1 AD inflammation was associated with an unidentified proteinaceous deposit.

*Conclusion*. Inflammation is associated with neuronal loss but is not specific for A-beta or tau. An unidentified proteinaceous deposit may also be crucial to the neurodegeneration seen in PS-1 AD. Understanding the neurotoxicity of this inflammatory response may lead to the development of therapies for many neurodegenerative disorders.

### 195P. Dehydroepiandrosterone Reduces Expression and Activity of BACE in NT2 Neurons Exposed to Oxidative Stress

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Recently, we showed that oxidative stress activates the expression and activity of β site AβPP cleaving enzyme (BACE), an aspartyl protease responsible of the β-secretase cleavage of AβPP. The identification of compounds able to prevent this event is an important goal of therapeutic strategies for Alzheimer's disease (AD). Dehydroepiandrosterone (DHEA) is an adrenal steroid that serves as a precursor of both androgens and estrogens. DHEA improves a variety of functions in the central nervous system. Moreover, a series of evidence suggest that DHEA displays antioxidant properties in different experimental models. In the present study we show that pre-treatment with DHEA is able to rescue the increase of BACE protein levels and the overproduction of APP C-terminal fragments, the direct products of β secretase cleavage, induced by oxidative stress in NT2 neurons. Moreover, DHEA results also able to prevent the oxidative stress-dependent increase of BACE mRNA, as shown by real time PCR analysis. The accumulation of amyloid β-protein (Aβ) soluble oligomers is a central event of the pathogenesis of AD. Thus BACE, being the enzyme that initiates the production of Aβ, is a drug target for AD. Our results imply that DHEA administration may slow down the AD pathological process, lowering Aβ accumulation.

## WORKSHOPS

### The Clinical Spectrum of Cerebral Small-Vessel Disease

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The traditional clinical manifestation of small vessel cerebrovascular disease has been lacunar stroke and so-called Binswangers disease. These are commonly due to hypertension although other vascular risk factors play a role. There are also a number of rare specific conditions affecting small vessels such as amyloid, CADASIL and some vasculitides. Lacunar stroke is recognised in some cases by the development of a classical lacunarsyndrome and in others by the general principles of comparing clinical deficit and the possible neurological structures involved with the cerebral arterial territories with a conclusion that only a small deep lesion could explain the findings.

However, this system only applies to eloquent structures, ie, those that produce deficits immediately recognisable by patients. More sophisticated imaging, especially MRI, shows that the majority of lacunarevents are silent. The primary manifestation of these lesions is subcortical vascular cognitive impairment. There is also an important interaction between degenerative dementias such as Alzheimer's disease and small-vessel cerebrovascular disease which appear to multiply together to accelerate cognitive decline.

Finally, there are white matter changes short of infarction, thought to be of ischaemic origin and termed leukoaraiosis. In the deep white matter leukoaraiosis may develop on the basis of diminished perfusion reserve coupled with episodic hypotension and ischaemia or transudation of neurotoxic plasma proteins through damaged vessel walls. These processes manifest themselves primarily through cognitive impairment, although the changes are subtle.

### Cerebral "Angiomyopathies" in Stroke and Aging

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Cerebral small vessel disease, especially affecting arterioles and (less often) capillaries is frequent in human subjects—2 common forms of this are cerebral amyloid angiopathy (CAA) and arteriosclerosis/ lipohyalinosis (AS/LH). Though both are commonly found with adancing age, AS/LH is, in many (if not all) instances associated with a history of hypertension, whereas (non-familial) CAA may occur sporadically but is often linked to Alzheimer disease (AD). Both AS/LH and CAA are associated with stroke, including cerebral infarcts and encephalic hemorrhage--the latter often a consequence of fibrinoid necrosis and/or microaneurysm formation on affected arterioles. CAA, when it involves arterioles, is characterized by degeneration and loss of medial smooth muscle cells (SMC), together with deposition of fibrillar Abeta amyloid protein. AS/LH, to the extent it can be considered a distinctive "lesion" of the cerebral microvasculature, is characterized by either SMC proliferation or degeneration, possibly both at different times during progression of the vasculopathy, with associated hyalinization of arteriolar walls that results from deposition of glycosaminoglycans and non-amyloid proteins. Thus, these 2 forms of microangiopathy are characterized by pathogenetic similarities and similar consequences, ie, brain hemorrhage and/or ischemic necrosis, though with a different topographic distribution. The ability to obtain relatively pure cultures of cerebral microvessel-derived SMCs allows for manipulation of these cells to study, for example, factors that potentially mediate overexpression of molecules of importance in progression of the different types of microvasculopathy, leading to stroke. (Supported by NIA Grants P50 AG16570 and P01 AG 12435).

### The Cerebral Amyloid Angiopathies, Microvascular Degeneration and Dementia

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Cerebral amyloid angiopathy (CAA) may co-exist in a wide variety of CNS sporadic disorders. Familial forms of CAA are rare but remain important in relation to the pathogenesis of cerebral haemorrhages and late onset dementias. Hereditary CAAs have certainly provided a better understanding of several novel amyloid protein precursors including cystatin C, amyloid β precursor protein (AβPP), prion protein (PrnP) and a novel gene ABri. Genotype-phenotype analysis suggests there is some relationship between the mutation cites and type and distribution of pathological lesions. Amyloid β (Aβ) type of CAA has been the most investigated in view of its enhanced presence in the elderly and in greater than 90% of Alzheimer's disease and Down's syndrome. In our series vascular pathology comprised intense deposition of fibrillar amyloid in walls of vessels in the leptomeninges, perforating arteries, intraparenchymal arterioles as well as focal deposits in capillaries associated with degeneration of both vascular smooth muscle and endothelium in vessel profiles. Intracerebral bleeds and lobar hemorrhages often occurred in advanced CAA, which was concomitant with ischaemic infarcts. Microinfarction and perivascular changes were frequently associated with small volume of infarction. We suggest vascular tone and blood-brain barrier function albeit locally are likely to be profoundly impaired in affected cerebral vessels. Evidence also suggests that angiopathy per se is a substrate for dementia.

Supported by grants from MRC (United Kingdom), Alzheimer's Research Trust (United Kingdom) and Alzheimer's Association (United States).

### CADASIL

Ruchoux M-M

Among CerebroVascular Diseases (CVD), CADASIL is an inherited angiopathy caused by mutations in the Notch3 gene. The pathological hallmark is systemic. Consequently, skin biopsy was used as a tool in CADASIL then in CVDs, revealing the existence of differents types of alterations.

A morphological skin vessel change classification was proposed and provided several informations: *i)* in CADASIL, VMCs destruction appears early and is likely to be the predominant factor, *ii)* similar skin vessel lesions appear to be related to the same biological modifications or were observed in patients belonging to the same family consequently new gene analyses are currently carried out, and *iii)* VMC alterations were observed in 80% cases and

an early major destruction of the VMCs were noticed in 54% cases before 50 years. Since VMCs secrete the most powerful endothelial permeability factor (VEGF), their alterations likely result in hypopermeability, in addition to a vessel wall hypotonia and a watershed hypoperfusion.

These data brought by morphological skin studies are currently confirmed with the discovery of a CADASIL model and cell culture experiments.

*Summary.* CADASIL provided a wealth of informations concerning CVDs: *i)* brain vessel alterations could be systemic, *ii)* skin biopsy could open new avenue for research, *iii)* new mechanism could be involved, and *iv)* new unknown diseases are now observed.

### Leukoaraiosis: Its Impact on Stroke and Disability

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*Background*. Leukoariosis (LA), appearing as bilateral, patchy, or diffuse CT low attenuation, or hyper-intense T2 MR areas in the subcortical white matter and expressing diffuse subcortical small vessel disease, frequently on hypertensive basis, has been reported to be associated with stroke, and with cognitive, motor, and mood disturbances, related to disability in the elderly.

*Methods*. *i)* Literature research and personal data related to the evidence about LA as predictor of either ischemic or hemorrhagic stroke. *ii)* Data from the LADIS (Leukoaraiosis And DISability) Project, European Union-supported Concerted Action involving 12 European centres, aiming at evaluating LA as independent determinant of transition to disability in the elderly.

*Results*. *i)* Evidence from either retrospective or prospective clinical/neuroimaging studies strongly support LA as independent predictor of both ischemic and hemorrhagic stroke. *ii)* In the LADIS Project, increasing frequency of heart failure, hypertension, diabetes, alcohol consumption, and previous stroke was observed across the 3 LA groups (from mild to severe). Worse performances along with increasing LA severity were apparent in: global functioning, motor performance, cognitive global functions, executive functions, and mood.

*Conclusion*. LA shares with stroke common pathophysiological mechanisms and, being likely an expression of the same disease, has to be regarded as an intermediate surrogate of stroke. In the elderly LA is independently associated with relevant functional impairment.

## PLATFORM PRESENTATIONS

### 196. In Vivo and Immunohistochemical Lesion Analysis of Axonal Damages in the White Matter of Binswanger's Disease

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We studied brain proton magnetic resonance spectroscopy (MRS) in clinically diagnosed Binswanger's Disease (BD) patients in order to investigate the N-acetylaspartate levels of the white matter (WM) lesions in each lobe as the in vivo molecular marker for axonal damage. Following that, WM lesions of the brains with clinicopathologically proven BD were evaluated topographically using a grading score by Klüver-Barrera and Bielschowsky stainings. The lesions were also immunohistochemically examined using molecular markers for axonal damage, amyloid precursor protein (APP) and encephalitogenic peptide (EP). Our results in the N-acetylaspartate levels in MRS as well as the pathological grading indicate that WM lesion in BD were significantly more prominent in the frontal periventricular and subcortical regions as compared to results from other subcortical WM lesions, in the order of the parietal, occipital and temporal lobulus. Moreover, the frequency of damaged nerve fibers labeled by the EP antiserum and APP immunoreactive fibers were significantly more prominent in frontal WM lesions in BD brains compared to control brains.

### 197. An Unusual Form of Fibromuscular Dysplasia of Extracranial Vertebral Arteries Causing Pontine Stroke in a 15-Year-Old Boy

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Fibromuscular dysplasia is a rare cause of childhood stroke. Diagnosis usually rests upon neuroimaging, and opportunities for histologic study are infrequent.

*Case Report*. An active, fit 15-year-old boy collapsed suddenly, the clinical picture rapidly developong into a "locked-in," syndrome. MRI of the brain and MRA of the head and neck vessels showed pontine and cerebellar infarcts and abnormal flow in the basilar artery suggesting thrombosis, but no evidence of dissection or dysplasia. Despite therapy with tissue plasminogen activator he died 2 weeks later. At autopsy the infarcts were confirmed, but detailed macroscopic examination of the cerebral vasculature did not reveal thrombosis, aneurysm or dissection. Microscopically, the circle of Willis was normal, but bilaterally the vertebral artery segments from within the intervertebral canal, and both intrapetrous carotid segments had thickened walls, focal intimal thickenings, extensive duplication of both internal and external elastic laminae, and medial dysplasia with marked infiltration of the muscle coat by mucopolysaccharide and numerous elastic fibers. Similar changes were present in the common, external and internal carotid vessels in the neck. The renal artery was not examined.

*Comment*. The unusual presentation in this adolescent, the anatomical location of the disease evading imaging investigation, emphasizes the need for morphologic investigation of arterial dysplasia, in this example revealing an unusual variant of medial fibromuscular dysplasia.

### 198. Vascular Abnormalities in Skin Biopsy Suggesting a Vascular Pathophysiology for Alternating Hemiplegia

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Alternating hemiplegia of childhood is a rare disease characterized by episodic paroxysmal attacks of hemiplegia involving either or both sides, associated with other paroxysmal neurological disorders. Hemiplegic attacks may suggest a vascular etiology. The pathophysiology remains unclear.

The use of skin biopsy to diagnose some vascular neurological diseases is now well established, like in CADASIL (Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) since the pathological hallmark is systemic.

The aim of the study was the research of vascular abnormalities in skin biopsy in patients with alternating hemiplegia.

*Methods*. Skin biopsy study was performed thanks to an ultrastructural analysis in 3 children with alternating hemiplegia.

*Results*. Interestingly, the 3 cases showed similar alterations. Firstly, the lumens were enlarged and the vessel walls were dramatically thin. Secondly, the endothelium presented vacuoles and stress bands. Thirdly, the most important alterations involved the media which displayed small and irregular shaped muscle cells showing vacuoles intracytoplasmic and some apoptotic nuclei. Some muscle cells were vanishing. Moreover, they were isolated from their neighbours since they lost their junctions. All of these features are likely to lead to a dramatically vessel wall weakness.

*Conclusion*. This finding could be related with probable vascular abnormalities in brain. We speculate a vascular pathophysiology for alternating hemiplegia. Vascular smooth muscle cells may the starting point of the vascular disease.

### 199. Minute Lineal Infarcts in the Territory of the Cortical Artery

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Minute infarcts in the cerebral cortex have been described insufficiently. We (FI et al, 2002) pointed out that there are such minute and vertical lineal infarcts as run along the cortical artery even in Japanese with somehow anatomical similarities with the lesions of so-called Viliuisk encephalomyelitis (VEM) in Siberia. We examined the details of these cortical infarcts in 146 cases which were selected from 170 consecutive autopsy cases in our hospital except for 24 cases of extensive cortical damage. The specimens including the cerebral cortex which had been taken for routine neuropathological examinations of each case were investigated. These characteristic cortical infarcts were observed in 45 (30.8%) cases. They coexisted with multiple cerebral infarcts due to atherosclerosis, thrombotic emboli and so on. They were lineal and extended ver-

tically within the cerebral cortex, and frequently occurred in the middle cerebral artery territory. Fresh infarcts showed some eosinophilic neurons which scattered in a narrow area extending to several layers in the cortex. Old infarcts revealed a lineal cystic lesion with mild fibrosis and evident gliosis, and a lineal gliotic lesion. We elucidate that the characteristic minute and lineal cortical infarcts, anatomically similar with VEM, occur in many Japanese cases probably due to occlusion of the cortical artery.

### 200. Vascular Pathology in CADASIL versus Cerebral Amyloid Angiopathy (CAA)

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*Objective*. In CADASIL and AD-associated CAA (AD-CAA) arteries are thickened and smooth muscle cells destroyed. GOM and connective tissue are deposited in CADASIL, but Aβ in AD-CAA. Small infarcts in WM and deep GM with sparing of cortex characterise CADASIL. In CAA intracerebral hemorrhages (ICH) are common.

*Patients and methods*. The sclerotic index (SI) in cortical and WM arterial walls and the integrity of BBB were analyzed in 4 CADASIL patients (R133C mutation), 4 familial (Δ9-PS1 mutation) and 10 sporadic AD patients, and 7 controls.

*Results*. In AD-CAA, SI of WM arteries were similar to those in controls. In CADASIL, SI in WM arteries were significantly higher than in cerebral cortex or in WM in controls and AD-CAA. In CADASIL, GOM and collagen type I but no Aβ were present in the thickened arterial walls. In AD-CAA mainly Aβ40 was deposited, whereas collagen was sparse. In CADASIL plasma proteins leaked from thin-walled cortical vessels but not from thick-walled WM vessels. In AD-CAA no major leakage was observed.

Conclusions: In CADASIL deep penetrating arteries are narrowed, whereas in AD-CAA they rather dilate and become fragile. This conforms with frequent infarcts and scarce ICHs in CADASIL and scarce infarcts and common ICHs in AD-CAA. In CADASIL WM lesions are ischemic, whereas in AD-CAA mainly secondary to neurodegeneration. Chronic vasogenic brain edema appears unlikely as major pathogenetic mechanism in CADASIL and AD-CAA.

### 201. Sporadic and Familial Cerebral Cavernous Malformations: Immunocytochemical and Ultrastructural Study

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*Introduction*. Cerebral cavernous malformations (CCMs) are typically made of capillary-type cavities. However, previous analyses concerning CCM lesions (Wong et al, 2000; Clatterbuck et al, Hoya et al, Uranishi et al, 2001) report several histological differences when comparing cavernomatous capillaries with normal cerebral vessels.

*Material and methods*. Paraffin samples of neurosurgery specimens were obtained from patients with sporadic CCMs or familial CCMs—Krit 1 genotyped patients—(7 cases each) and from control brain tissue. In addition, epon-embedded samples from 2 sporadic and 2 familial cavernomas were studied. Immunocytochemical studies used antibodies to *a*. von Willebrand factor, *b*. CD34, and *c*. smooth muscle α-actin, myosin heavy chain and smoothelin (respectively markers of *a*. endothelial cells, *b*. endothelial cells and pericytes and *c*. smooth muscle cells).

*Results*. Strong immunocytochemical positivity was observed with anti von Willebrand factor, CD34 and smooth muscle α-actin. Endothelial cells showed the presence of gaps and scattered tight junctions, numerous intracytoplasmic vesicles and large amounts of haemosiderin. The basal membranes were multilaminated. Subendothelial cells were rare, often limited to scarce cytoplasmic remnants.

*Conclusions*. The characteristics of cavernomatous vessels are different from those of continuous-type capillaries found in normal cerebral vessels.

### 202. The β-chemokines MCP-1 and MIP-1α Enhance Adhesion of CD4+ T Cell Subsets to Human Brain Microvessel Endothelial Cells In Vitro

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*Background*. Chemokines have been implicated as mediators of CNS inflammation. The role of β-chemokines in leukocyte recruitment across the blood-brain barrier (BBB) remains poorly understood. We investigated the effects on T cell (TC) adhesion of the β-chemokines, MCP-1 and MIP-1α, which are produced and released by human brain microvessel endothelial cells (HBMEC) in vitro.

*Methods*. Primary HBMEC cultures grown to confluence on permeable collagen membranes in a double chamber treated with TNF-α (100 U/ml) and IFN-γ (200 U/ml) for 24 hours. MCP-1 (50-500 ng/ml) or MIP-1α (10-100 ng/ml) were placed in the lower chamber to establish concentration gradients. TC were placed over HBMEC and incubated for 1 hour at 37°C.

*Results*. Adhesion of resting and naive TC to unstimulated HBMEC was minimal. Cytokine activation of EC upregulated adhesion by 4-fold. TC activation resulted in further increase in adhesion to resting and activated HBMEC. Neither chemokine had any effect on the adhesion of resting or naive TC to unstimulated or cytokine treated HBMEC. In the presence of MCP-1 and to a lesser extent MIP-1α gradients, there was significant increase in activated TC adhesion to cytokine treated HBMEC. These findings suggest a possible role of MCP-1 and MIP-1α in the recruitment of CD4+ TC subsets across the BBB in CNS inflammation.

## POSTERS

### 203P. Time-Distant Morphological Changes in Ischemic Stroke

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*Background*. Most experimental and clinical papers concerning cerebral ischemia concentrate on acute phase of stroke. Scarcely of data referring to time-distant changes compel us to examine morphological picture in late period after the ischemic insult.

*Material and methods*. We evaluated on light microscopy 10 brains of patients who died from one month to 14 years after ischemic stroke. On tissue slides routine histological stainings and immunohistochemical reactions with antibodies against lectins: Ulex Europeaus, Triticum Vulgaris and Badirea Simplicifolia were applied.

*Results*. Except postapoplectic lacunas white matter spongiosis was found. Intensity of the spongiosis decreased with increasing distance from the lacunas. Immunohistochemical study of blood vessels in damaged white matter revealed swelling of the endothelial cells, their invagination into the vessel lumen and segmental lack of immunoreactivity within vessel wall. Changes were observed independently on the presence or not arterial hypertension in patient's history.

*Conclusions*. Progressing damage of the white matter after ischemia may be caused not only by degeneration of axons of neurons destroying by the stroke but also by pathological changes in small blood vessels, especially in capillaries. Hence, vascular leukoencephalopathy probably is not only the consequence of arteriolar damage but also microangiopathy.

### 204P. Cerebral Blood Flow in Patients at the Early Recovery Period After Stroke

Mirzadjanova Z

Stroke is an actual medical and social problem both for progressive and developing countries. Therefore rehabilitation of stroke patients is a very important problem. Ethiopathogenic therapy at the early recovery period after stroke mainly depends on hemodynamic particularities.

We investigated blood flow velocity by Doppler technique in extra- and intracranial arteries in 70 patients within one year after acute stroke before and after treatment.

We revealed Doppler-signs of stenosis in the internal carotid arteries in 19 patients; 27.1% (8 on the right side and 11 on the left side). Blood flow asymmetry in intracranial arteries was determined in 48 patients. A low flow velocity in vertebral arteries was revealed in 47 patients (67.1%). We didn't find strong relationships between internal carotid artery stenosis and intracerebral blood flow deficit. It can be explained by good cerebral autoregulation in most part of these patients.

All patients were divided into 3 groups: *i)* 23 patients whom performed sequent refractory and supravascular laser therapy (SRSLT); *ii)* 25 patients whom performed laseropuncture (LP); and *iii)* 22 patients whom performed acupuncture (AP).

TCD-data after SRSLT therapy have shown increase of liner blood flow velocity (BFV) in all intracranial arteries, mostly in PCA (on 21.1%, $p<0,05$ on the right side) and (on 17.05%, $p>0,05$ on the left side). LP also led to increasing of BFV, but not in all intracranial arteries, mainly in ACA and partially in MCA.

Influence of (AP) on intracerebral hemodynamic was minimal. Thus, SRSLT and LP lead to improvement of cerebral hemodynamic in patients at the early recovery period after stroke and can be recommended with complex drug therapy.

### 205P. Multiparemetric Study on the Mechanisms of Slowly Evolving Cerebral Infarction after Transient Cerebral Ischemia

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Different from permanent cerebral ischemia, transient cerebral ischemia induces slowly evolving cerebral infarction. We investigated the relationships among tissue energy failure, MRI finding and behavioral change in the slowly evolving cerebral infarction.

*Method*. Mongolian gerbils were subjected to 2 times 10-minute occlusion of the left common carotid artery. ADC map and T2 map were generated from MRI. Brains were removed for imaging of tissue ATP content and tissue succinic dehydrogenase (SDH) activity. Behavioral tests for motor, somatosensory and visual dysfunction were performed.

*Results*. Tissue ATP content and SDH activity slowly decreased in the cortex and dorsolateral of caudate and thalamus over 2 days after ischemia. The change paralleled to the increase of T2 and decrease of ADC. Behavioral tests revealed close correlations between the severity of motor, somatosensory and visual dysfunction and the infarct size of the corresponding cortices.

*Conclusion*. Transient cerebral ischemia induces slowly evolving energy failure and brain edema associated with cerebral infarction. Neurological deficit coordinates to the evolution of irreversible injury in the corresponding cortex.

### 206P. Endogenous Brain-Derivated Factors in Hemorrhagic Stroke Treatments

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The problem of efficacy of treatment of stroke and of hemorrhagic stroke particularly is still actual now.

The novel Cerebral® remedy belongs to the group of brain-derived factors and its therapeutic action in cerebrovascular disorders treatment is in the process of intensive exploration. The efficacy and mechanism of total remedy and revelation of active compounds of Cerebral® action are the goals of the present study. The peculiarity of our novel Cerebral® remedy is in that the last is produced from the brain of the animals, successfully survived after experimental stroke. The ratio of free acids to peptides of Cerebral® differs from those one of the other medicines of that family.

The exploration of separate compounds of Cerebral® obtained by HPLC method revealed the active fraction of the remedy. In the experiments of bilateral hemorrhagic stroke of internal capsule anterior limb in rats this fraction gave the best outcome judged by histopathological indices, neurological deficit score, restoration of locomotor, exploratory and skilled forelimb activities.

The influence of active fraction as well as total remedy activates the synthesis and secretion of NGF, elevates the survival in acute hemorrhagic stroke conditions and accelerates the recovery.

## WORKSHOPS

### Patterning the Brain

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In humans, formation of the complex brain structure occurs over months of prenatal development. During this period, through the patterning of progenitor cells in the proliferative zones, neuroepithelial cells are instructed to undergo proper proliferation, migration, differentiation and connectivity. Relatively mild abnormalities in any of these steps are often associated with marked alterations in brain function. Our research focuses on the identification, characterization and expression profiling of genes controlling such processes.

A collection of cDNAs that are expressed from 5- to 60-fold in the embryonic mouse telencephalon (E14.5) compared to adult telencephalon, and mainly corresponding to novel genes, has been isolated through subtractive hybridization, and arrayed on glass coated slides.

The array of subtracted cDNAs is currently used to perform expression-profiling experiments on neural progenitor cells, and mouse mutants. Further characterization of these differentially expressed genes will include over-expression/inactivation in cells and brain-slices. We strongly believe that these studies will enable the selection, identification and characterization of a new repertoire of genes involved in telencephalic development as well as putative candidates for neurological disorders.

### LIS1 and Dynein Motor Function

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The current understanding of the process of mammalian neuronal migration has resulted primarily from neurobiological studies of brain development in normal mammals and mutant mice such as *reeler*. These studies provided important insight into the laminar development of the CNS, and have provided critical entry points into these pathways, while genetic and cell biological studies have guided our understanding of the function of these gene products in neuronal migration. For example, cloning of the gene mutated in *reeler* and the identification of its protein product RELN led to the elucidation of a RELN-dependent signal transduction pathway. Other genes have been identified that are responsible for neuronal migration defects in the human (LIS1 and DCX) and mouse (Cdk5 and its required activators p35 and p39), but until recently the relationship between these genes involved in migration were unknown.

Several recent studies have demonstrated that LIS1, the protein product of a gene mutated in the human neuronal migration defect lissencephaly, binds to and regulates dynein motor function in the cell. These studies place LIS1 in the midst of a well-studied motor complex important for several critical cell functions, and perhaps provide a context for integrating several neuronal migration pathways. In this presentation, we will provide some background on the human and mouse studies that provided the entry points to neuronal migration pathways, the investigation of gene function in model systems and what these studies tell us about the mechanisms regulating neuronal migration. We will focus on the recent studies of LIS1 function in animal models, and how they can potentially integrate other known pathways of neuronal migration.

### A Dual Role for Mash1 in Generation of Neurons and Oligodendrocytes in the Postnatal Brain

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Progenitors in the subventricular zone of the postnatal telencephalon generate neuronal precursors migrating rostrally to the olfactory bulb, as well as oligodendrocytes and astrocytes. It is currently not known whether mechanisms that govern cell fate specification in the embryonic telencephalon also operate for postnatal progenitors. The proneural bHLH protein *Mash1* has an essential role in neurogenesis in the embryonic telencephalon. Here we show that *Mash1* is also expressed in the postnatal subventricular zone. Most *Mash1*-expressing cells have characteristics of transit amplifying progenitors for the olfactory neuron lineage, while a smaller population expresses markers of oligodendrocyte precursors. We have addressed the role of *Mash1* in these lineages by examining *Mash1* mutant mice at birth when they die. Olfactory bulbs in mutant mice have a drastically reduced number of neurons and oligodendrocytes. To determine whether this phenotype reflects a role of *Mash1* in specification of neuronal and oligodendrocyte lineages, we generated neurosphere cultures from *Mash1* mutant subventricular zone progenitors. Differentiating mutant neurospheres produced much fewer neurons and oligodendrocytes than control cultures, while the number of astrocytes was unchanged. Our results thus indicate that *Mash1* has a central role in specification of both neuronal and oligodendrocyte precursors, in a common lineage for GABAergic interneurons and oligodendrocytes in the postnatal brain.

### Glial Progenitor Migration in the Developing Forebrain

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In the mammalian CNS, for the most part, the wave of gliogenesis follows that of neurogenesis, and the great majority of glial cells are generated in the perinatal period from progenitors in the subventricular zone (SVZ). We have investigated the migration pathways and dynamics of progenitors from the neonatal rat forebrain SVZ by labeling them in vivo with recombinant retroviruses encoding cDNA for fluorescent proteins and visualizing the movements of living cells by time-lapse video microscopy in slice preparations. Cells migrated radially and tangentially after emigration into white matter, cortex, and striatum. During migration, elongation of the leading process and nuclear translocation were independent or linked. Orthogonal turning involved either cessation of cell body movement and formation of a new leading process or continuous cell body movement and bending of the leading process. A small number of glial progenitors migrated tangentially across the

corpus callosum, presumably in conjunction with unmyelinated axons, to colonize the contralateral hemisphere. Such dynamics contribute widespread distribution of glia. At this point, it has not been clear about specific molecular cues that could characterize the glial progenitor migration. A better knowledge of glial progenitor development and migration may give important clues to the understanding of pathomechanisms of human malformations and glial neoplasms.

## PLATFORM PRESENTATIONS

### 207. Type III Lissencephaly: A Spectrum of Neuro-Ectodermal Dysplasia?

Allias F; Buenerd A; Attia-Sobol J; Dijoud F; Clémenson A; Bouvier R; Encha-Razavi F

Lissencephaly is absence or rarefaction of cerebral convolutions. Two major groups, type I and type II are classically described. A third type (LIS III) with distinct neuropathological pattern was first described in Neu-Laxova syndrome (NLS) and recently reported by our group in an apparently different context of Fetal Akinesia Deformation Sequence (FADS), with thick and/or fragile skin, but no ichthyosis (OMIM# 601160).

We found LISIII in 7 unrelated fetuses presenting with FADS, microcephaly/lissencephaly, delayed cerebral cortex maturation, lack of callosal and cerebrospinal tracts, neuronal depletion of the germinal zones, basal ganglia, brain stem nuclei and spinal cord. Changes suggestive of a neurodegenerative process, such as balloon shaped neurons with chromatolysis and fragmentation were found. Thick and fragile skin without ichthyosis was noticed in 2 cases.

Our hypothesis is that LIS III is the common denominator between NLS and OMIM# 601160. A detailed analysis of NLS discloses evident similarities between the two entities, that may be both linked to a "neuro-ectodermal dysplasia." Polyhydramnios, flexion deformity of the limbs, and oedema constantly reported in NLS are part of FADS. On the other hand, skin abnormalities although less severe are often found in OMIM# 601160. Further studies are needed to clarify the frontier between the 2 entities and the possibility of a lesional continuum between them.

### 208. Neuropathology of Prenatally Detected Cerebral Malformations: Radio-Anatomical Correlations

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We report the results of the neuropathological study of 87 prenatally detected cerebral malformations. We consider for each case: the term of ultrasound malformation detection, the type of detected sign (ventricular dilatation, posterior fossa abnormality, neural tube defect [NTD], arhinencephaly, corpus callosum agenesis, microcephaly, gyral defects), the associated fetal malformations, the familial, maternal and obstetrical antecedents.

Our data reveal that: *i)* the earlier a malformation is detected, the more frequently it has a genetic origin; *ii)* some malformations are detected during the whole pregnancy, such as ventricular dilatations, others are detected only during the second trimester (absence of cerebral structures such as vermis or corpus callosum agenesis) or later (cerebral growth disturbances: microcephaly, pontocerebellar hypoplasia, gyral defects); and *iii)* for one ultrasound abnormality, the neuropathological diagnosis differs according to the term, ie, a ventricular dilatation is mostly related to defects of cerebral construction (NTD, Fowler syndrom, Walker-Warburg,) during the first trimester of pregnancy, and to a rhombencephalic dysplasia or an acquired lesion from the second trimester .

Our study demonstrated the major place of the neuropathological study in the diagnosis assessment of prenatally diagnosed cerebral malformations. It also emphasises the key role of the term in diagnosis assessment.

### 209. Cortical Damages* Following Inhibition of Monocarboxylate Transport During Development

Adle-Biassette H; Baud O; Gressens P; Evrard P; Hénin D; Verney C

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*Aim*. In a former work was emphasized the prenatal presence of monocarboxylate transporters* in the developing rodent cerebral cortex. Here we investigated the effects of inhibition of transport of monocarboxylate, including lactate on different stages of cortical development in mice using alpha-cyano-4-hydroxycinnamate (4-CIN)*.

*Material, methods and results*. Intraperitoneal injections of 4-CIN in an alcoholic solution or in DMSO at high dose of 200 mg/kg induced a too high rate of maternal and fetal mortality. We used lower doses (100 mg/kg daily) during late gestational ages (embryonic day (E)17, E18, E19). The pups sacrificed on postnatal days (PD) PD0, PD1, PD2 showed an hypotrophy associated with cortical lesions as microgyria. Postnatal intraperitoneal injection of 4-CIN, 80 mg/kg daily over PD1-PD3 and sacrifice at PD5 induced apoptosis in the cortex without clearly detectable cytoarchitectural lesions. Following prenatal injection BRDU labelled neurons at PD10 were significantly decreased in the cortex of mice who received 4-CIN compared to matched controls.

*Conclusion*. The pattern of cortical lesions induced by 4-CIN varied according to the developmental stage. It could be mediated by different mechanism, ie, alcohol neurotoxicity and/or secondary to 4-CIN. This latter is known to alter the neuronal energetic metabolism by blocking the transport of lactate, the main energy supply of neurons provided by astrocytes.

### 210. Early, Progressive Disruption of the Glia Limitans Leads to Cerebellar Dysgenesis in Dystroglycan-null Mice

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Conditional deletion of brain dystroglycan leads to granule cell migration errors in the cerebellar cortex (*Nature* 418:422-425). Studies of postnatal development in a second model of brain dystroglycan deletion, nestin-Cre/dystroglycan-null mice, show early and progressive disruption of basal lamina at the cerebellar glia limitans. Disruptions are already present on postnatal day 2. Glia limitans pathology is accompanied by structural abnormalities in Bergmann glia, gliosis at the cerebellar surface, and fusion of adjacent folia. Multifocal clusters of external granule cells fail to migrate. These data suggest that dystroglycan is required for maintenance of the basal lamina of the developing cerebellar glia limitans. The disruption of this basal lamina appears to be responsible for cerebellar cortical dysgenesis in these mice.

### 211. MAP2 and MAP1B Expression in Ibotenate-induced Neuronal Migration Disorder in Hamsters

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*Purpose*. To investigate the cytoskeletal changes in neuronal migration disorders, immunohistochemical expressions of MAP2 (high-molecular weight form) and MAP1B were examined in brain lesions induced by ibotenate in hamsters.

*Materials and methods*. Intracerebral injection of a one microgram ibotenate was performed to the newborn hamsters. Histological and immunohistochemical examinations were carried out in the brains 1, 2, 3, 5, and 7 days after injection.

*Results*. The cortical lesions observed after ibotenate injections had a strong resemblance to the following neuronal migration disorders: *i)* microgyria, *ii)* subcortical nodular heterotopia, and *iii)* leptomeningeal glioneuronal heterotopia. MAP2 and MAP1B expressions were observed throughout the cortical plate into the molecular layer, showing strong dendritic staining. Small number of MAP1B immunoreactive neurons were also detected in the intermediate and ventricular zones. Dendritic processes of the neurons in microgyria or leptomeningeal glioneuronal heterotopia showed immunoreactivity for MAP2, as well as MAP1B. Subcortical nodular heterotopia included strong immunoreactive MAP2-positive neurons, but MAP1B immunoreactive elements were sparsely distributed.

*Conclusion*. We concluded that the expression of high-molecular weight form MAP2 is up-regulated in the structural remodeling of neuronal migration disorder, especially in the formation of subcortical nodular heterotopia.

## POSTERS

### 212P. Periventricular Leukomalacia is Associated with Progenitor Cell Deficiency

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Periventricular Leukomalacia (PVL) is an important cause of cerebral palsy and intellectual impairment in immature infants. In 60 cases of PVL we have observed a lesion not only in the white matter but also a deficit of portions of the ependyma. Because the causative insult for PVL (ischemia, cytokines, toxicity, etc.) occurs during a time when neurogenesis, gliogenesis, cellular migration and differentiation are occurring we questioned whether the effect of this disruption of the ependyma was associated with a lesion in the underlying germinal matrix. We examined the defective ependyma and persisting periventricular germinal matrix in these brains using Mushashi-1 and Nestin, markers expressed in neural progenitor cells including stem cells. Musashi-1 is a neural RNA-binding protein involved in the regulation of cell fate determination, neuronal glial and ependymal differentiation and maintenance of the stem cell state.

Nestin marks neuronal and glial precursor cells. In the normal immature brain isolated ependymal cells and germinal matrix cells expressed both markers. In PVL the expression of both Musashi-1 and Nestin was less than normal. We hypothesize that in PVL, particularly in very low birth weight infants the germinal matrix is interrupted by PVL or its generating factors so that the pool of progenitor cells is reduced with detrimental effects on both the developing white matter and cortex in survivors with PVL.

### 213P. Congenital Perisylvian Polymicrogyria: Neuropathological Study of 5 Fetal and Neonatal Cases

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Congenital Perisylvian Polymicrogyria (CPP) is a cortical malformation involving sylvian fissure and frontoparietal operculum, responsible for epilepsy and mental retardation in children. The diagnosis is based on clinical and radiological criteria. Neuropathological correlations are few.

*Patients*. We studied 3 neonates after neonatal death and 2 fetuses from medical pregnancy termination. All were male. MRI was performed in the 2 fetuses and in one neonate. All patients had post-mortem examination including neuropathological examination.

*Results*. MRI demonstrated abnormally thickened cortex with enlarged sylvian fissure in the 3 cases. History examination emphasized familial history of stillbirth and neonatal death (1), parental consanguinity (1). Karyotype disclosed a partial duplication 1q in a case. Neuropathological examination showed a polymicrogyria mainly unlayered, bilateral (4) or unilateral (1), with different degrees of cortical involvement associated with an abnormally thick corpus callosum (1), and significant neuronal loss in the anterior horn of the spinal cord (1). Brain lesions were associated with arthrogryposis (1), IUGR (1), visceral and extremities anomalies.

*Conclusion*. Our findings demonstrate that: (1) CPP includes a wide spectrum of phenotypic expression, (2) some cases have a genetic cause consistent with X-linked inheritance in agreement with the recently demonstration of a linkage to the Xq28 region, (3) a genetic heterogeneity is suggested by a chromosomal anomaly and a consanguinity in 2 cases, (4) MRI allows a prenatal diagnosis.

### 214P. Antenatal Hydrocephaly Combined to Adductus Thumbs, Corpus Callosum and Pyramidal Tracts Hypoplasia or Agenesis is Associated with L1 Mutations

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*Background*. Severe childhood hydrocephaly, in male patients associated with other development defects including corpus callosum agenesis has been linked to L1 mutation. Therefore, we analysed this gene in a series of 56 newborns and fetuses presenting hydrocephaly and corpus callosum abnormality.

*Methods*. The entire coding region of the L1 gene was screened either by fluorescence assisted mismatched analysis or denaturating high performance chromatography. Each mutation detected was characterised by genomic sequencing. Genetic results were correlated to anatomo-clinical data.

*Results*. Mutation of L1 gene was observed in 37 patients (66%). In 12 of them (32%), it involved the extracellular region of the protein that corresponds either to the IgG domains or the fibronectin binding site. Pathological examination revealed in all patients with a L1 mutation, the presence of adductus thumb(s),

hypoplasia or agenesis of corpus callosum and pyramidal tracts. Aqueduct stenosis was found in 35 cases (94%).

*Conclusions*. L1 mutation is a mechanism frequently involved in the pathogenesis of ante-natal hydrocephaly associated with combined adductus thumb(s), corpus callosum and pyramidal tract hypoplasia or agenesis.

### 215P. A Report on 6 Cases of Rhombencephalosynapsis: An Abnormal Neural Tube Dorsal Patterning Due to Teratogenic Agent(s)?

Marcorelles P; Chabaud JJ; Collet M; Sonigo P; Lagarde N; Gonzalès M; Encha-Razavi F

Rhombencephalosynapsis (RS) is a rare congenital hindbrain malformation, characterized by agenesis (or extreme hypoplasia) of the vermis, fusion across the midline of cerebellar hemispheres and dentate nuclei. No specific clinical presentation is described, but prognosis is poor in fetal cases discovered on hydrocephalus.

We report on 6 fetuses with normal karyotype, from 16 to 32 weeks GA, in which severe hydrocephalus was the main sign discovered on ultrasound and leads to termination of pregnancy. RS was most of the time a neuropathological finding, associated in half of cases with others supratentoriel midline abnormalities, including fused superior and inferior colliculi (mesencephalosynapsis, MS), and fused thalami (diencephalosynapsis, DS).

This spectrum of congenital defects may be linked to abnormal patterning of the rhombencephalon, mesencephalon and diencephalons. The role of the Isthmic Organizer (IO) in the patterning of dorsal mesencephalon and rhombomere 1, through Fgf8 is well established. However, the etiology of RS-MS-DS remains unknown. A few familial cases are described, but RS is usually sporadic and the karyotype normal. A possible link with teratogenes has been suggested. A recent increasing of RS-MS-DS in our series, leads us to wonder about a possible link with teratogene(s). Further studies are needed to confirm this view.

### 216P. Link between Neurogenic Fetal Akinesia and Chromosome 21?

Martinovic J; Maurin Ganne ML; Tantau J; Esculpavit C; Benacki A; Vekemans M; Morichon N; Encha-Razavi F

Fetal Akinesia Deformation Sequence (FADS) refers to a spectrum of abnormalities linked to decreased/lack of intra-uterin fetal movements. The pathogeny of FADS is heterogenous and may be neurogenic, myogenic or due to restrictive dermopathy, and intrauterin constraint. Neurogenic FADSs (N-FADS) have in common spinal motor neuron alterations, isolated or associated with brainstem and/or cerebral abnormalities. Classically, neuronal alterations are linked to extrinsic (hypoxic-ischemic, toxic) or intrinsic (neurodegerative) factors. However, etiologic factors are rarely identified.

In a male fetus of 17-week GA with N-FADS, a detailed neuropathological study disclosed motor neuron and cranial nerve nuclei alterations. Cytogentic analysis found interstitial deletion of chromosome 21q, involving the Down Syndrome Critical Region (DSCR).

In 21q-syndrome, arthrogryposis is a frequent finding. Our hypothesis is that DSCR may contain motor neuron survival gene(s). Further studies are needed to confirm this view.

### 217P. Lissencephaly with Agenesis of Corpus Callosum and Rudimentary Dysplastic Cerebellum: Studies on a Subtype of Lissencephaly with Cerebellar Hypoplasia (LCH)

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We present a detailed neuropathologic description of an autopsy brain from a 7-day-old neonate born at 38-gestational weeks, presenting with a possible subset of lissencephaly with cerebellar hypoplasia (LCH). The brain was severely hydrocephalic and totally agyric. The corpus callosum was absent and deep gray matter structures indistinct. A rudimentary dysplastic cerebellum, dysplastic olivary nuclei and nearly complete absence of corticospinal tracts were also noted. Microscopic examination revealed various types of dysplastic and malformative features throughout the brain in addition to the classic four-layered neocortical structure characteristic of type I lissencephaly. Unique features in the present case were: *i)* bilateral periventricular undulating cortical ribbon-like structures (PUC) mimicking fused gyri and sulci, *ii)* large dysplastic neocortical neurons positive for phosphorylated neurofilament, calbindin-D28K, tuberin, hamartin, LIS1, reelin and Dab1, *iii)* derangement of radial glial fibers, and *iv)* heterotopic gray matter composed exclusively of granule cells in the cerebellar deep white matter. The clinicopathological features in the present case are suggestive of a distinct category of lissencephaly with cerebellar involvement. We suggest a possible classification of this unique case among the LCH syndromes.

### 218P. Apoptosis Regulation and BCL-2 Expression in Human Fetal Cortex

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*Background*. Apoptotic natural cell death and it's regulatory proteins are likely to be important in "sculpting" the developing fetal cortex. To date there are few studies in human cortex.

*Aim*. To study the expression of the apoptotic regulatory proteins BCL-2, and P53, as well as cyclinD1 and ki-67 in autopsy sections of human foetal cortex, and correlate with the anatomic and temporal patterns of distribution.

*Design*. Paraffin blocks were selected from 5 cases which underwent neuropathologic assessment. Gestational age ranged from 22 to 35 weeks. All cases had peri-ventricular germinal matrix hemorrhage associated with prematurity but no cerebral malformation or infarcts. Standard immunohistochemistry(IHC) was performed on full thickness cerebral hemisphere sections and correlated with LFB/HE morphology.

*Results*. BCL-2 expression was seen in 60% of cases in developing cortical mantle zone. Positivity was seen in neuroepithelial cells and associated linear processes. At 22 weeks expression was in deeper mantle zone while at 25 to 35 weeks there was strong band-like expression in the superficial mantle cells. Germinal matrix was immunonegative, as were all areas for cyclinD1, Ki-67 and P53. No correlation was seen with apoptotic cellular morphology.

*Conclusion*. This study supports a dynamic regulation of apoptosis during cortical development. Altered expression of apoptotic

regulatory proteins may be more extensive than indicated by morphology. There is a suggestion of a specific spatio-temporal patterning of expression of the apoptosis inhibitory protein BCL-2 in developing cortex. Inhibition of neuroepithelial cell apoptosis at critical points may be necessary for normal cortical development.

### 219P. Is Apoptosis of Heterotopic Progenitors in Fetal Cerebellum Programmed Differently than that of Orthotopic Progenitors?

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*Material*. Cerebella of human fetuses of various ages were examined for histological identification of apoptotic heterotopic progenitor cells (single cell pattern). Floccular dysgenesis (2 cell pattern) was also investigated as a comparison.

Fetuses were legally terminated due to trisomy or other malformation syndromes or were born by spontaneous abortion without malformations except the incidental heterotopia in the cerebellum.

*Method*. Immunohistochemistry by antisera against the caspase-3 and p85-PARP, in addition to the TUNEL method, was used to detect apoptosis. Expression of bcl-2 and CD68 was examined for the recognition of apoptosis inhibition and macrophage mobilization, respectively.

*Results and Discussion*. Heterotopic progenitors of single cell type in the cerebellar nuclei displayed an accelerated rate of apoptosis accompanied by mobilization of macrophages in comparison to those of the transient subpial granular cell layer. This may well be the reason why differentiated granular cell heterotopia in the cerebellar nuclei has never been observed.

Floccular dysgenesis, on the contrary, displayed no accelerated apoptosis rate, which may explain why this dysgenesis of 2 cell type is frequently found in adult patients with trisomy 21 as well as incidentally in other patients.

*Conclusion*. Heterotopic progenitor cells in the human cerebellum might be differently programmed from orthotopic progenitor cells for apoptosis, although local environmental influences cannot be excluded. Additionally, we speculate that 2 cell pattern heterotopia might be resistant to apoptosis.

### 220P. Acute Effects of Antenatal Betamethasone Treatment on Glucocorticoid Receptor Density in Fetal Sheep Brain

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HPA maturation and its function can be permanently programmed during fetal life and may predisponate to several adult diseases. However, it is unclear how brain glucocorticoid receptors (GR) are involved in such events. The aim of this study was to evaluate whether betamethasone (BM) down-regulates GR in the fetal sheep brain and whether there are differences at various BM treatment regimes.

Pregnant sheep were treated with BM i.m. or directly to the fetal jugular vein or with an equal amount of saline at 40, 110 and 128 days of gestation (dGA) in doses similar during antenatal glucocorticoid therapy. GR were estimated using a monoclonal antibody against GR (Clone BuGR2).

GR were found in neurons and glial cells at 110 and 130 dGA but not at 40 dGA. BM infusion directly to the fetus led to down-regulation of GR in the hippocampus and subcortical white matter at 110 but not at 130 dGA. Maternal BM administration showed no effect. In conclusion, fetal but not maternal BM administration at the dose used clinically in premature labor may lead to an immediate down-regulation of GR in specific brain regions before the endogenous surge of cortisol.

### 221P. Fukuyama Congenital Muscular Dystrophy (FCMD)

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To verify the hypothesis that the pathological findings in Fukuyama congenital muscular dystrophy (FCMD) is caused by the hypoglycosylation of alpha-dystroglycan (α-DG) due to defects of fukutin gene, we compared the distribution of fukutin and α-DG in developing and adult mouse tissues. In the central nervous sysem, antisera against fukutin and α-DG both labeled the migrating neurons of developing cerebral and cerebellar cortex, and the pontine migratory stream. In adult mice, fukutin and α-DG were extensively co-expressed in neurons of the cerebral and cerebellar cortex, hippocampus, basal ganglia and olfactory bulb, as well as in the pontine nucleus and the cranial nerve nuclei. As for the somatic organs, fukutin and α-DG were also immunopositive in the acinar cells of pancreas, the renal glomerulus and tubular cells, and the epithelium of the bronchi, salivary gland, alimentary tract and skin in both fetal and adult mice. We further examined immunohistochemically the glycosylation status of α-DG in autopsied FCMD cases (n = 5) and found evidence of hypoglycosylation in the hippocampal neurons, and the epithelium of the kidney, lung, skin and intestine of FCMD. These support the hypothesis that fukutin is involved in the glycosylation process of α-DG, and the defect of this process plays an essential role in the neuropathology of FCMD. The reason why the involvement of muscle and brain predominates in FCMD remains unclear, but the physiological function of α-DG in each organ should be further explored.

## Primary CNS Lymphoma

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Primary central nervous system lymphoma (PCNSL) have recently receivedconsiderable clinical attention due to their increasing incidence. However, their molecular pathogenesis is still largely unknown. In order to define the histogenetic origin of these tumors, PCNSL from HIV-negative patients were analyzed for immunoglobulin gene rearrangements. Clonal IgH gene rearrangements were demonstrated in all cases. There was a remarkable biased usage of the V4-34 gene segment, while there was no preferential usage of D(H), J(H), V(kappa), J(kappa), V(lambda), or J(lambda) gene segments. The tumor cells had introduced somatic mutations into their functionally rearranged Ig gene segments with a pattern indicating selection of the tumor cells for expression of a functional antibody. The mean mutation frequencies for the heavy and light chains were remarkably high exceeding other lymphoma entities. Furthermore, there was evidence for ongoing mutation. Additional interphase cytogenetic analysis demonstrated recurrent chromosomal translocations in PCNSL with breakpoints within the IGH and IGK locus. In addition, there were breakpoints in the BCL6 locus; gains of 18q21 represented the most frequent genetic imbalances. Interestingly, while PCNSL share similarities with extracerebral DLBCL with respect to the presence and frequency of IGH translocations, they appear to differ in the usage of translocation partner genes. Taken together, these studies indicate that the tumor cells of PCNSL have been experienced a germinal center reaction and that the process of somatic hypermutation may be of pathogenetic significance for lymphomagenesis. In addition, IGH translocations appear to play a role in their molecular pathogenesis.

## Interfaces Between Intracranial Tumors and Genetics: Meningiomas

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Despite nearly a century of study, the classification of meningiomas remains a work in progress. The current WHO scheme recognizes 13 separate variants and 3 malignancy grades. Meningioma was one of the first solid tumors characterized by a karyotypic aberration, monosomy 22. Since that time, great progress has been made in further genotyping, with associations to histopathology and biologic behavior. Since meningiomas are common in NF2, it is not surprising that the NF2 gene (22q12) was the first gene implicated in familial and sporadic meningiomas. Some data suggest that loss of the protein product, merlin is associated with fibroblastic/transitional, rather than meningothelial histopathology. Although the precise function of this protein 4.1 family member is not known, its tumor suppressor role is established, given that reintroduction into deficient meningioma cell lines suppresses growth and the recently developed leptomeningeal conditional Nf2 knockout mouse develops meningiomas. The role of other candidate genes is less certain, though there is data to suggest that other protein 4.1 family members may also be involved. In terms of malignant progression, cytogenetic models have been created based on associations with tumor grade. Implicated regions include losses of 1p, 3p, 6q, 9p, 10, and 14q, and gains of 17q and 20q. For most, candidate genes have yet to be identified, though the loss of the p16 gene region on 9p21 is common in anaplastic meningiomas, particularly the subset with shorter survival. New strategies, such as high-throughput gene expression profiling are currently being utilized to identify additional targets of interest.

## Hemangioblastoma and VHL

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Hemangioblastomas of the CNS are highly vascularized tumors that occur in patients with hereditary von Hipple-Lindau disease (approximately 25% of cases) or sporadically. Since VHL loss of function has also been demonstrated in sporadic hemangioblastomas, loss of function of the VHL gene appears to be a causative underlying mechanism of hemangioblastoma development.

Current evidence suggests that the stromal cells represent the neoplastic component of the tumour, whereas the capillary network formes as a consequence of aberrant gene expression in the stromal cells. The stromal cells express high levels of the hypoxia-inducible transcription factors (HIF) –1 and –2. Constitutive HIF expression in stromal cells is a consequence of increased stability of this proteins. HIF-1 and –2 are rapidly degraded by an oxygen-dependent process involving several enzymes, transcription factors and the proteasomal complex. In addition, proteosomal degradation of HIF-1 and –2 is dependent on functional pVHL. In stromal cells, the inactivated pVHL leads to HIF-1 and –2 accumulation. As a consequence, HIF target genes such as vascular endothelial growth and erythropoietin are upregulated on the transcriptional level, explaining the vascular and cystic phenotype of the tumors and the high incidence of erythrocytosis in patients with VHL disease.

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## PLATFORM PRESENTATIONS

### 222. Immunophenotypic Spectrum of Benign Meningiomas. A Study of 98 Consecutive Cases

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In order to differentiate meningiomas from other mesenchymal tumors of the meninges or to predict their biological behavior, many immunohistochemical studies have been reported with conflicting results. To determine the immunoprofile of benign meningiomas 98 consecutive cases have been tested with the antibodies EMA, S100, AE1/AE3, CD34, HHF35, factor XIIIa, p53, bcl-2, and MIB-1. There were 75 females and 23 males. Ages ranged from 19 to 86 years (mean: 50.8 years). The tumors were classified as transitional (43), meningothelial (35), fibrous ( 12), microcystic (3), papillary (2), psamomatous (1), secretory (1), angiomatous (1). EMA was positive in 89.25%, S100 in 28.12%, HHF35 in 7.22%, CD34 in 6.32%. AE1/AE3 was positive in 3 cases (one fibrous, one meningothelial and one secretory). bcl-2 was positive in only one meningothelial tumor. Factor XIIIa and p53 were negative in all cases. The mean MIB-1 value was 1.64% ± 2.1% (range: 0-10.6%). Both papillary tumors scored 2.4% and 2.8%, respectively. There was no statistical significance between the antibodies expression and the different histological types. CD34 which may differentiate solitary fibrous tumors of meninges from fibrous meningiomas was positive in only one out the 11 examples of the latter. The consistent negativity for bcl-2 and for p53 in our series differs from some data of the literature where the immunostaining of these antibodies are generally positive albeit in a widely variable percentage. Prognostic significance based on these apoptotic markers should be taken with caution.

### 223. Expression of MMP-2 and MMP-9 in Benign, Atypical and Malignant Meningiomas

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Milano, Italy.

*Background*. Matrix metalloproteinases (MMPs) are zinc dependent proteolytic enzymes that degrade extracellular matrix and several lines of evidence indicate that they play an important role in the invasiveness and proliferation of tumors. The aim of this work was to verify whether differences in MMP-2 and MMP-9 expression could be detected between benign, atypical and malignant meningiomas.

*Methods*. We performed an immunohistochemical study using a monoclonal antibody against MMP-2 and a polyclonal antibody against MMP-9 on 15 benign meningiomas, 28 atypical and 4 malignant meningiomas.

*Results*. Immunoreactivity for MMP-2 was found in all meningiomas examined and was localized in the cytoplasm of neoplastic cells, with nuclear sparing. Only a few cells in focal areas were immunodecorated in benign meningiomas, while in most atypical and malignant meningiomas the antibody recognized a larger number of neoplastic cells throughout the tumor.

No difference between benign, atypical and malignant meningiomas was observed with MMP-9 antibody that stained intensely the cytoplasm of neoplastic cells diffusely in the tumor. Moreover MMP-9 immunoreactivity was also found in macrophages infiltrating necrotic areas of malignant meningiomas.

*Conclusions*. Our findings suggest that overexpression of MMP-2 may be correlated to aggressive behaviour in meningiomas and could be useful for diagnosis and possible therapeutic strategy of these tumors.

### 224. Immunohistochemical Localization of E- and N-cadherins, Beta-catenin and EMA in Menigiomas

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Our study examined cadherin-catenin adhesion in meningiomas having various histological forms and mostly benign biological behaviour. We wished to clarify how cadherin-catenin adhesion affects invasion, metastasis, and morphology of tumor cells and histological architecture(forms) of tumors.

Analysis of immunohistological localization of E- and N-cadherins and beta-catenin in 38 meningiomas relate appearance, histological forms, and tumor cell morphology. Results indicate that cadherin-catenin adhesion affects the formation of various histological variants and the morphology of tumor cells.

Meningioma immunoreactivity: E-cadherin = 31.6% (12/38); N-cadherin = 17.4% (4/23); Beta-catenin = 95.5% (21/22); EMA = 47.8% (11/23). E- cadherin tumor cell variant immunoreactivity: Transitional = 28.6% (2/7); Fibrous = 16.7% (1/6); Meningothelial = 42.9% (6/14); Angiomatous = 33.3% (1/3); Psammomatous = 20% (1/5); Atypical = 0% (0/1); Malignant = 0% (0/1); Papillary = 0% (0/1). N- cadherin tumor cell variant immunoreactivity: Transitional = 0% (0/3); Fibrous = 25% (1/4); Meningothelial = 12.5% (1/8); Angiomatous = 0% (0/3); Psammomatous = 33.3% (1/3); Atypical = 0% (0/1); Papillary = 100% (1/1). Beta-catenin tumor cell variant immunoreactivity: Transitional = 100% (3/3); Fibrous = 100% (4/4); Meningothelial = 87.5% (7/8); Angiomatous = 100% (3/3); Psammomatous = 100% (3/3); Atypical = 100% (1/1). EMA tumor cell variant immunoreactivity: Transitional = 0% (0/3); Fibrous = 75% (3/4); Meningothelial = 50% (4/8); Angiomatous = 33.3% (1/3); Psammomatous = 66.7% (2/3); Atypical = 0% (0/1); Papillary = 100% (1/1).

### 225. Clinicopathological Role of Rhabdoid Cells in Meningiomas

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The presence of rhabdoid cells in meningiomas indicates a malignant transformation and poor prognosis. When there is at least a focal area of rhabdoid cells in the meningioma tissue, it can be designated as a rhabdoid meningioma, which belongs to Grade III by the criteria of the WHO classification 2000. Rhabdoid morphology was identified in 4 cases within 214 meningiomas. Three cases were recurrent within 5 years after the first operations. In 2

recurrent cases, tumors were composed of rhabdoid, papillary and atypical components. In another recurrent case, tumor consisted of rhabdoid and atypical parts. The fourth case that received radiotherapy after the first operation had no recurrence for 2 years. The tumor comprised rhabdoid, papillary, chordoid, clear cell and atypical patterns. Rhabdoid cells that had immunoreactivity for vimentin and epithelial membrane antigen were identified in small parts of the tumors. Other histological features (papillary or atypical) were major tumor components in our cases. Clinical role and histogenesis of rhabdoid cells in meningiomas remains unclear. We suggested that the rhabdoid cell was one of the additional features in atypical or papillary meningiomas, and that those meningiomas should be termed as not rhabdoid meningiomas, but atypical (anaplastic) or papillary meningiomas with rhabdoid component.

### 226. Diagnostic and Prognostic Role of Ki-67 Proliferation Index in Human Meningiomas

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*Background*. Histopathology alone cannot always predict the clinical behaviour of human meningiomas. The aim of the present study was to assess the diagnostic and prognostic significance of proliferative activity in these tumors by means of different Ki-67 antibodies.

*Methods*. Paraffin sections of thirty-four human meningiomas graded according to the latest WHO criteria (nine benign, sixteen atypical, and nine anaplastic meningiomas), were incubated with four different Ki-67 antibodies (MIB-1, Immunotech; NC-MM-1, Novocastra; NC-Ki-67p, Novocastra; rah-Ki-67ag, DAKO).

*Results*. There were statistically significant correlations between proliferation indices (PI) obtained by each of the Ki-67 antibodies ($p<0.05$). The Ki-67 PIs increased with increasing tumor grade and distinguished significantly between benign and atypical/anaplastic meningiomas ($p<0.05$). However, the Ki-67 PIs did not discriminate between the atypical and anaplastic forms. Increased levels of Ki-67 PIs were associated with higher risk of tumor recurrence, but these results did not reach statistical significance ($p>0.05$).

*Conclusions*. Ki-67 antibodies may be helpful in tumor grading and as a prognostic marker for patients with meningiomas. However, further studies are needed to fully clarify the clinical role of Ki-67 in these tumors, and a reliable marker to identify meningiomas with a more aggressive behaviour remains to be found.

### 227. Correlation Between Histological Grade and MIB-1 and p53 Immunoreactivity in Meningiomas

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*Objective*. Meningiomas are slow-growing benign tumors; however, complete removal can be difficult and recurrence is an issue. A variety of clinical, radiologic, and pathologic prognosticators are important in determining the biological behavior of meningiomas. In this study, we investigated all histological parameters related to recurrence including brain invasion and the correlation between histological grade, MIB-1 proliferation index and p53 protein expression.

*Material and methods*. Sixty biopsy specimens of meningiomas, including 48 grade I, 11 grade II, and one grade III, were examined immunohistochemically using the monoclonal antibodies MIB-1 (Ki-67) and p53 protein. Histopathological diagnosis of meningioma was confirmed in each sample based on the criteria of the latest WHO brain tumor classification.

*Results*. Seven out of 60 meningiomas revealed brain invasion regardless of their grade. The mean MIB-1 proliferation index (PI) values were 1.1% for the grade I, and 2.3% for the grade II. p53 protein expression was found in 54.1% of the grade I, and 72.7% of the grade II. An MIB-1 PI of 6.7% and a p53 immunoreactivity of >50% were identified in grade III meningioma. Our results indicate that in meningiomas, a statistically good correlation exists between histological grading, MIB-1 and p53 protein expression ($p<0.01$). However, we found no association between brain invasion and histological grade, proliferative activity and p53 expression.

*Conclusion*. MIB-1 and p53 protein expression may be used as additional criteria to establish grade, especially in the evaluation of borderline meningiomas. Although the number of the cases is limited in our study, brain invasion should be considered as a prognostic parameter independent from grade, MIB-1 and p53 immunoreactivity.

### 228. Fine Structural and Immunohistochemical Studies Including Those of E- and N-Cadhrins and Beta-Catenin of a Papillary Meningioma with 5 Recurrences

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Papillary meningioma is a rare form of meningioma with characteristic papillary architecture and exceptional malignancy. Because this condition is so rare, little is known of the fine structure of its tumor cells or immunohistochemical properties of its cadherins (likely important in intracellular adhesion and histological architecture), catenins (cadhrins-binding proteins attached to intracellular actin filaments which contribute to cellular morphology), and other cellular proteins.

Using surgically obtained fifth recurrence right fronto-temporal tumors from a 74-year-old man, we report details of the structure of tumor cells and immunohistochemical localization of E- and N-cadhrins, beta-catenin, apithelial membrance antigen (EMA), glial fibrillary acid protein (GFAP), vimentin, factor 8, and Ki-67 (MIB-1).

Histologically, tumor cells showed basically perivascular patterns of papillary and solid arrangements. Multi-nucleated cells of varying size and type were common. There was little mitosis or necrosis. Endothelial proliferation was marked in some areas.

Structually fine, intercellular digitation was simpler than fourth recurrence digitations. Various intercellular adhesion structures were present but not prominent.

Immunohistochemically, many tumor cells are strongly reactive for N-cadherin, indicating an important role in intercellular adhesion in papillary meningioma. Some tumor cells are reactive for EMA, but unreactive or obscure for beta-catenin, GFAP, vimentin, and factor 8. About 10 to 30% of tumor cells are immunoreactive for Ki-67 (MIB-1).

## POSTERS

### 229P. Immunohistochemical Expression of Microglia in Secretory Meningioma

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*Background*. Recent technical developments have made available better methods to identify microglial cells, the resident immune cells of the central nervous system. However, the exact role of microglia in meningiomas is still unknown. It was suggested that the mononuclear cells infiltrating brain tumors may play a role in the secretion of substances such as growth factors and cytokines. Secretory meningiomas (SM) are a rare histological subtype of meningiomas, surrounded by more peritumoral edema than usual, out of proportion to the size and presumably secondary to a secretory-excretory phenomenon.

*Methods and results*. We have investigated the immunohistochemical of major histocompatibility complex (MHC) class II molecules (CR3/43) and of the microglia/macrophages marker CD68 in 6 paraffin-embedded SM. CD68 and CR 3/43-immunoreactivity was detected on the inside of cell membranes and in the cytoplasma of morphologically heterogeneous mononuclear cells distributed over the tumor parenchyma and interstitial tissue as scattered single cell or groups of cells.

*Conclusions*. Microglia in SM are well equipped to function as antigen presenting cells. We speculate that CD68 and CR 3/43-positive cells may play important roles, such as secretion of growth factors, regulation of the tumor growth and immunogenicity and probably also in secretion of various substances related to surrounding brain edema.

### 230P. Immunohistochemical Study of Anaplastic Meningioma with Special Reference to the Phenotypic Change of Intermediate Filament Protein

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*Aims*. The phenotypic changes in the transformation of classic or atypical meningioma to anaplastic meningioma were investigated.

*Methods*. Among 9 patients with anaplastic meningioma 4 men and 5 women aged from 32 to 75 years, 4 cases were identified as anaplastic meningioma at the first operation (de novo type). While, 5 cases were identified as classic or atypical meningioma at the first operation but at recurrence had transformed to anaplastic meningioma (secondary type). Immunohistochemical analysis was performed with the ABC method using monoclonal antibodies for GFAP , cytokeratin, alpha-internexin, NFPs (70 kd, 168 kd, and 200 kd), desmin, vimentin, CD34, Ki-67, EMA, and S-100 protein.

*Results*. Immunohistochemical analysis revealed positive immunoreactivity for cytokeratin, alpha-internexin, neurofilament proteins, vimentin, and glial fibrillary acidic protein during the course of progression. Expression of epithelial membrane antigen decreased with malignant progression. Marked expression of cytokeratin was observed in anaplastic meningioma. Ki-67 labeling index increased at every recurrence of both the de novo and secondary types.

*Conclusion*. The major phenotypic changes in the transformation of meningioma from the classic to the anaplastic type are loss of meningioma architecture, decreased expression of epithelial membrane antigen, increased expression of vimentin, and metaplastic expression of alpha-internexin and neurofilament triplet proteins.

### 231P. Simultaneous Occurence of Glioblastoma Multiforme and Meningioma. A Case Report

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*Introduction*. Multiple primary intracranial tumors of different histological type not due to radiotherapy and phacomatosis are rare.

*Clinical presentation*. A 56-year-old woman presented with a 5-week history of cephalgia. Three months before admission to our hospital in 1999, the patient had experienced a generalized tonic clonic seizure. Upon presentation, neurological examination was unremarkable. A MRI scan revealed a right parieto-occipital tumor of the brain, measuring $4 \times 3$ cm and intracranial tumor of the left ponto-cerebellar angle, measuring $1.6 \times 2.5$ cm, arising from tentorium. She underwent two craniotomies followed by focal irradiation of the parieto-occipital region. In 2002 a MRI scan demonstrated a recurrence of tumor in the right occipital lobe, measuring 3 cm in diameter. A right parieto-occipital recraniotomy was performed.

*Neuropathological findings*. Histologically, the intraparenchymal tumor was hypercellular with pleomorphic astrocytes. Mitoses, necrosis, and vascular endothelial proliferation confirmed a diagnosis of glioblastoma multiforme. Furthermore, histological findings of the intracranially located tumor indicated a spindle cell tumor showing a fascicular arrangement with whorling and psammoma bodies. A diagnosis of meningioma was made.

*Conclusion*. Much speculation remains regarding the origin of multiple tumors of different histological type. Both meningiomas and gliomas are relatively common, therefore their concurrence is probably coincidental. On the other hand, meningioma or glioma can stimulate the adjacent brain parenchyma or arachnoid cells into neoplastic proliferation. However, for tumors in different hemispheres, chance alone is applicable.

### 232P. A Case of Probable Metastatic Brain Tumor Mimicking Olfactory Groove Meningioma

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A 55-year-old homeless male was admitted to our hospital for obtunded consciousness in the road on August 23, 2002. CT disclosed a large tumor in the right frontal base crossing the midline and accompanied with marked perifocal brain edema. The tumor was interpreted as meningioma arising in olfactory grooves because

enhanced CT showed homogeneous enhancement and angiography revealed late tumor stain fed with ophthalmic arteries. On August 29, the soft and suckable extra-axial tumor was removed totally. Histological examination disclosed highly cellular tumor consisting of small and irregularly ovoid tumor cells making nests demarcated with fibrovascular stroma. The tumor had many cells under apoptosis and mitosis. Apparent rosette formation was absent. Papillary arrangement was equivocal, but we reached a diagnosis of atypical or anaplastic meningioma. As differential diagnosis olfactory neuroblastoma, hemangiopericytoma or meningeal PNET were noticed. After tumor removal the patient recovered to restore daily activity, but the right neck lymph node swelled rapidly. A needle biopsy was made on September 30, and showed the same histology as the meningeal one. Small cell type carcinoma had to be considered. The patient disappeared from our hospital on October 5 and further evaluation became impossible. Histological findings suggest the meningeal tumor was not intrinsic but derived from metastasis of poorly differentiated carcinoma with neuroendocrine differentiation because of positive immunostain for synaptophysin.

### 233P. Malignant Solitary Fibrous Tumor (SFT) of the Meninges with Ribosome-Lamella Complex

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A 76-year-old man died after the surgical removal of a meningeal tumor that compressed the spinal cord at the C7 level. Seventeen years earlier a meningeal tumor in the posterior fossa was removed. A local recurrence was removed 13 years after the first operation.

The autopsy revealed 4 meningeal tumors surrounding the brain stem in addition to a local recurrence in the left cerebellar hemisphere.

*Microscopic examination*. Tumor invasion of the right inferior colliculus. All tumors were composed of spindle cells arranged in fascicles between prominent eosinophilic bands of collagen. There were no whorls or psammoma bodies. Mitoses were almost absent in the first biopsy as well as in the tumors disclosed at autopsy. The first recurrence and the spinal tumor showed at least 7 mitoses pr. 10 HPF. Necroses were never observed.

*Immunohistochemistry*. Strong expression of CD34 and bcl-2. No expression of EMA or S-100 (among others).

*Electronmicroscopic examination*. Intracytoplasmatic ribosome-lamella complexes. Collagen fibrills in the extracellular compartment. No basement membrane-like material.

*Diagnosis*. Malignant solitary fibrous tumor of the meninges.

*Discussion*. The prognosis of most meningeal SFTs is excellent. This case illustrates that the biologic behaviour is unpredictable even in the absence of malignant histologic features. The ribosome-lamella complex is often observed in haematopoietic malignancies. To our knowledge they have never been described in SFTs.

### 234P. Neoplastic Meningitis: a Clinico-Pathological Study of 3 Cases

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*Objective*. Neoplastic meningitis is the result of multifocal seeding of the leptomeninges by malignant cells and is pleomorphic in its clinical presentation. We investigated clinico-pathological study in this disease.

*Patients and methods*. Three cases of neoplastic meningitis were performed clinical and neuroimaging studies, laboratory analysis of cerebrospinal fluid (CSF), antemortem CSF cytology, and autopsy.

*Results*. *Case 1*. A 53-year-old woman had a headache and weakness of her both legs. The patient had been healthy until 2 years earlier when the diagnosis of a squamous cell carcinoma of the uterine cervix was made. CSF protein was elevated and glucose level was low, and cytology was positive for malignant cell consistent with neoplastic meningitis of a squamous cell carcinoma of the uterine cervix. The patient was treated with radiotherapy and intrathecal chemotherapy. *Case 2*. A 72-year-old woman had weakness of her left leg and multiple cranial nerve palsies. Post-mortem examination confirmed the diagnosis of neoplastic meningitis with lymphoma. *Case 3*. A 50-year-old woman presented with headache and subacute dementia. Radiological examination disclosed lung cancer with thoracic vertebral body metastasis.

*Conclusion*. CSF examination is one of useful laboratory test in diagnosing neoplastic meningitis. Cytology is very helpful when positive for malignant cells, in supporting a diagnosis and also in following response to treatment.

## WORKSHOPS

### A Role for Astrocyte Derived Glutathione in Delaying Neuronal Mitochondrial Damage and Neurodegeneration

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Increased generation of reactive nitrogen species, by astroglial cells, has been proposed to be a potential factor for the loss of mitochondrial function associated with the neurodegenerative process. Cell culture studies reveal that when neurones are exposed to nitric oxide/peroxynitrite loss of activity of one or more of the components of the mitochondrial electron transport chain (ETC) is observed. Furthermore, such damage appears to proceed neuronal cell death. In contrast, astroglial cells when exposed to comparable conditions appear relatively resistant. Factors influencing this differential susceptibility may include a superior intracellular concentration of glutathione (GSH) and an ability to maintain cellular energy demands by up regulating glycolytic flux. In order to create a more appropriate model system, we have cultured astrocytes and neurones together. Under such conditions, neuronal susceptibility to reactive nitrogen species appears to be diminished. Studies have shown that the neuronal GSH content is enhanced by the trafficking of GSH from astrocytes to neurones. Recently, we have found that nitric oxide increases GSH biosynthesis in astrocytes, but not neurones, leading to enhanced GSH release. Furthermore, the released GSH may be protected from oxidative degradation in the extracellular environment by the concomitant release of a factor with properties comparable to superoxide dismutase. Failure of this GSH trafficking may compromise the neuronal antioxidant reserves leading to increased vulnerability of the ETC and cell death.

### Insights into Mechanisms of Cell Damage and Death in Parkinson's Disease: Expression Profiling with Microarrays

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*Introduction*. Alpha-synuclein accumulation in the form of Lewy bodies (LBs) and Lewy neurites (LNs) is the new histopathological diagnostic criterion for Parkinson's disease (PD) and other disorders that were previously grouped under the general heading of Lewy body disease (*http://www.icdns.org*). The clinical phenotype of these diseases is heterogeneous and presently includes PD, PD with late dementia (PDD), and dementia with LBs (DLB).

*Methods*. We are using U133 oligonucleotide microarrays (Affymetrix) to establish a comparative expression profile of the substantia nigra and other brain regions in PD and related disorders. The Human Genome U133 Set (HG-U133A and HG-U133B) is comprised of 2 gene chips containing over 1 000 000 unique oligonucleotide features representing more than 33 000 human genes. Neuropathological cases are carefully selected for their clinical and histopathological features and tissue pH>6.5.

*Results and discussion*. Microarray data are characterized by very high dimensionality. As a first step, we have applied conventional classification techniques that best distinguish between PD and control groups. In addition, we perform a comprehensive analysis of variation in gene expression relative to the severity of disease as reflected by conventional histopathological phenotype, eg, the number of Lewy inclusions and the severity of neuronal cell death, the extent of alpha-synuclein immunoreactivity and the level of microglial activation. This requires the application of statistical estimation procedures that employ "gene modelling" against the various data sets. The absence of classical apoptosis from the substantia nigra in PD calls for a rigorous assessment of neuronal cell death pathways to identify disease specific mechanisms.

### Neuronal Damage During Viral Infection

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Viral infections of the nervous system are commonly limited to the meningeal space but may spread to involve the brain parenchyma. Brain infection depends upon viral access to the nervous system, binding to a surface receptor and a receptive intra-cellular environment to permit viral replication. As an encephalitis, viruses elicit neuronal damage through a wide variety of mechanisms. Some viruses directly infects neuroglial elements causing damage from cell dysfunction to cell lysis. Each viral-host infection involves a uniquely evolved pathway. New emergent infections accent the rapidity with which this relationship can evolve.Beyond acute infections many viruses mediate a more protracted infection of the nervous system and lead to damage through less clear pathways. Some viral infections are never cleared (eg, herpes and paramyxoviruses) opening the possibility for chronic non-lytic infection of nervous system cells to persist and continue to mediate neuronal dysfunction and damage. Most viral infections are limited by the immune response. In the ideal situation this implies eradication of the infectious agent. Unfortunately, if the virus replicates rapidly, before the immune system mounts an adequate anamnestic response, substantial neuronal damage occurs. Worse, in some viral infections, a dysregulated immune response itself will lead to self directed neuronal damage. Frequently viral host evolution has led to a compromise where the host contains but does not eradicate the virus at the benefit of more limited host damage from the immune response.

### Neuronal Death in Brain Ischemia in Man

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Several interrelated processes are involved in the death of ischemic neurons. These include depletion of oxygen and high-energy metabolites, peri-infarct depolarisation, oxidative injury and inflammation. The consequences vary according to the contributions of these processes, and may be energy failure and necrosis, or the activation of caspases and apoptosis. Activation of caspases after brain ischemia is largely consequent on mitochondrial release of cytochrome c, but Smac/DIABLO, lipid peroxidation, reduction in protein synthesis, and activation of cyclin-dependent kinases may also contribute. Experimental studies of brain ischemia

have mainly used young, healthy animals without underlying vascular disease, and models of ischemia-reperfusion injury that maximize oxidative stress and apoptosis. In contrast, most ischemic strokes affect in patients with atherosclerotic disease and, except in cases of rapid thrombolysis or resuscitation after cardiac arrest, rarely involve significant reperfusion. Human autopsy studies of focal infarcts suggest that apoptosis makes only a very limited contribution to the neuronal degeneration, even though many ischemic neurons show aberrant entry into the cell cycle. Inhibitors of apoptosis are therefore unlikely to be useful in most focal brain infarcts in man, but other observations indicate that blocking of neutrophil adhesion to endothelium may be of benefit. Therapeutic interventions in stroke patients will require detailed evaluation using a range of outcome measures. Some experimental studies suggest that inhibition of neuronal apoptosis after brain ischemia may not necessarily salvage function or do more than delay neuronal loss.

## PLATFORM PRESENTATIONS

### 235. Oxidative Damage to Neuronal RNA Shows Age-dependent Increases in Human Brain

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*Background*. We have reported that an oxidized nucleoside, 8-hydroxyguanosine (8OHG), a biomarker of oxidative RNA damage, is significantly increased in vulnerable neurons of age-associated neurodegenerative disorders. In this study, we have investigated whether the levels of oxidative RNA damage in neurons show age-dependent changes among individuals without dementia or other neurological disorders.

*Methods*. Postmortem tissue of the hippocampus or the temporal neocortex was obtained from 50 control cases ranging in age from 0. 3 to 86 years. The presence of 8OHG was identified immunocytochemically in tissue sections, and relative density of 8OHG immunoreactivity was measured with an image analysis system.

*Results*. A moderate or faint 8OHG immunoreaction was observed in the neuronal cytoplasm in presenile and senile cases, while 8OHG was virtually undetectable in younger cases. As a function of age, relative density of neuronal 8OHG immunorectivity increased significantly in the hippocampal subiculum ($n=27$, $r=0.55$, $p<0.01$) as well as in the inferior temporal/occipitotemporal gyrus ($n=23$, $r=0.42$, $p<0.05$). Neither postmortem intervals nor causes of death were significantly associated with the levels of 8OHG immunoreactivity.

*Conclusions*. These results show for the first time that there is an age-dependent increase of oxidative damage to RNA in neuronal cells of human brains.

### 236. Neuroprotection Against Excitotoxocity and Ischemia Using Cell-Permeable Peptides that Inhibit JNK Action

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Activation of c-Jun N-terminal kinase (JNK) occurs in excitotoxicity and ischemia. We here report that cell-penetrating peptide inhibitors of the JNK pathway protect neurons against both these insults.

In dissociated cultures of cortical neurons, NMDA (100 μM) provoked a rise of JNK activity to a near-maximal level in 30 minutes followed by a decrease. We used L- and D-peptides containing a cell-entry sequence from HIV-TAT and a sequence from JIP-1/IB1 to prevent interaction between JNK and many of its targets. Both peptides totally prevented the toxic effects of NMDA, kainate or anoxia and the associated caspase-3 activation and morphological changes.

In vivo, we tested the more stable D-peptide. It penetrated the blood-brain barrier and gave strong protection in 2 models of middle cerebral artery occlusion. In adult mice, intraventricular administration as late as 6 hours post-occlusion reduced the lesion volume by more than 90%, a protection that was maintained for at least 14 days and was accompanied by behavioral sparing. In 14 day rat pups with permanent occlusion, systemic peptide delivery 6 or even 12 hours post-ischemia still protected. Protection correlated with prevention of increases in c-Jun activation and c-fos transcription.

### 237. Neurotrophic Effect of Exogenous Microglia on Ischemic Hippocampal Pyramidal Neuron

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*Background*. We have been reported that exogenous microglia enter the brain parenchyma through blood brain barrier from the circulation and migrate to the ischemic pyramidal neurons. In this study, we investigated the effect of the exogenous microglia on the ischemic pyramidal neurons.

*Methods*. Fluorescently labeled microglia were injected into the artery of gerbils 24 hours before or 24 hours after the transient forebrain ischaemia. At seven days after the ischemia, the behavioral task (passive avoidance task) was performed, then the brains were removed and, the number of surviving hippocampal pyramidal neurons were counted. The mechanisms of microglial neurotrophic effect also were investigated.

*Result*. Fluorescently labeled microglia migrated to the ischemic hyppocampal layer. Injection of microglia caused not only prolongation in response latency in passive avoidance task, but also increase the survival pyramidal neurons, even if the injection was performed 24 hours after ischemia. Pretreatment of microglia with interferon gamma enhanced microglial neuroprotective effects. Western blot analysis and sensitive sandwich immunoassay showed that the increase of the expression of brain derived neurotrophic factor and glial cell line derived neurotrophic factor in the homogenate of hippocampus with the injection of microglia.

*Conclusion*. This study represents the first experimental demonstration of neurotrophic effect of microglia on the transient forebrain ischemia in vivo. Microglia may have a potential to be used as a candidate for the cell therapy for central nervous system repair following transient global ischemia.

### Coexistence of Necrosis and Apoptosis in Exofocal Postischemic Neuronal Death

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Both necrosis and apoptosis have been reported to be involved in the postischemic neuronal death. However, little is known about the mechanism of exofocal postischemic neuronal death (EPND).

*Method*. We examined the time course of magnetic resonance image and ultrastructure of the substantia nigra (SN) after ipsilateral middle cerebral artery occlusion (MCAO) in rats.

*Results*. MRI revealed a transient increase of T2 value associated with decrease of apparent diffusion coefficient, signifying transient cytotoxic edema, in the SN at 4 days after onset of the ipsilateral MCAO. Ultrastructural examination at 2 to 4 days after MCAO revealed many neurons with hydropic swelling and a few neurons

with typical apoptotic nuclear changes in the SN. Number of surviving neurons in the SN was significantly reduced 7 days after MCAO.

*Conclusion*. EPND evolves in the SN after MCAO in rats. Predominant mechanism of EPND in the SN is necrosis. Coexistence of apoptosis with necrosis in the SN indicates heterogeneous mechanism and/or variable phenotype of EPND.

## POSTERS

### 239P. GADD34 Expression in Human Brain After Cardiac Arrest

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*Background*. GADD34 is a growth arrest and DNA damage inducible gene upregulated in response to DNA damage, cell cycle arrest and apoptosis. GADD34 shares a region of homology with herpes simplex virus neurovirulence protein ICP34.5. In vitro, ICP34.5 precludes host cell protein synthesis shutdown after virus infection. It is believed that GADD34 also modulates protein synthesis and therefore may play an important role in the brain after ischemia.

*Aim*. To determine if GADD34 is upregulated in the human brain after cardiac arrest.

*Materials and methods*. Ischemic damage was assessed using H&E and GADD34 expression assessed immunohistochemically in the cortex and hippocampus of subjects surviving <24 hours (n = 11) or 24 hours to 7 days after cardiac arrest (n = 11) and in age-matched controls (n = 15). Ischemic damage and GADD34 staining was assessed using a scoring method with investigators blinded to category and data analysed using a Mann-Whitney statistical test.

*Results*. Faint cellular GADD34 staining was present in control tissue. GADD34 staining was markedly increased in selectively vulnerable regions in the 24 hour to 7 day survival group. GADD34 was present in neurons displaying ischemic cell change and in some morphologically normal neurons. A correlation was found between GADD34 expression and the extent of ischemic damage (*$p<0.05$). Double labelling revealed co-localisation of GADD34 and PCNA (proliferating cell nuclear antigen) in many neurons.

*Conclusions*. GADD34 is upregulated in the brain after cardiac arrest and may perform an important function in resolving protein synthesis inhibition and DNA damage repair.

### 240P. Time Dependent Mitochondrial Mediated Programmed Brain Cell Death in Sepsis

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*Introduction*. Apoptosis is a possible mechanism of brain dysfunction occurring in septic syndrome.

*Materials and methods*. We report an experimental study of 112 rats that were subjected to sepsis by cecal ligation and puncture. Sham-operated animals (n = 20) underwent the same procedure but without ligation or puncture. Eighty-two septic animals were randomly subdivided into 6 groups,studied in 6, 12, 24, 36, 48, and 60 hours after the operation. Fifty septic rats were used as the survival group. Morphologic features of apoptosis were evaluated via electron and light microscopy. Immunohistochemistry was performed on paraffin-embedded brain sections for bax,bcl-2,cytochrome-c and caspase-8. In septic rats apoptosis was detected in neurons of the CA1 region of the hippocampus,choroid plexus and in Purkinje cells of the cerebellum in hematoxylin-eosin stained specimens.

*Results*. Electron microscopy showed condensation of the nuclei and compact masses of chromatin in neurons of septic rats. A high linear relationship of bax immunoreactivity and time of death was observed characterized by upregulation of bax in the early stage of septic syndrome,progressively decreasing in the late phases ($p = 0.001$), the same for cytochrome-c ($p = 0.001$). Animals expressing cytochrome-c in neurons had favorable prognosis compared to cytochrome-c negative animals($p=0.02$). Bcl-2 and caspase-8 expression was in standard levels in all the time evaluated.

*Conclusions*. There is evidence that neurons undergo apoptosis during sepsis,leading progressively to their dysfunction. Time dependent Bax activation and less caspase-8 seem to be the pathways to programmed brain cell death in sepsis and appears to favor prognosis of septic subjects.

### 241P. Cytoprotective Effect of Epigallocatechin Gallate on Phosphoinositide 3-kinase/Akt and Glycogen Synthase Kinase-3 Pathway in Oxidative-stressed PC12 Cells

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Neurodegenerative diseases (ND) are associated with oxidative stress, and antioxidants including epigallocatechin gallate (EGCG) have been tried as a potential therapeutic regimens of experimental model of ND. We performed this study to determine the neuroprotective role of EGCG on up stream and down stream signals in oxidative-stress-injured PC12 cells by exposing to $H_2O_2$.

Following 100 $\mu$M $H_2O_2$ exposure, the viability of EGCG pretreated cells was evaluated by MTT assay. Expression of cytochrome c, caspase-3, poly (ADP-ribose) polymerase (PARP), PI3K/Akt and GSK-3 was examined by Western blot.

EGCG or z-VAD-fmk pretreated cells showed increased viability. Dose-dependent inhibition of caspase-3 activation and PARP cleavage was demonstrated by the pretreatment of each agents. On the other hand, inhibition of cytochrome c release was detected only in EGCG pretreated cells. EGCG pretreated cells showed decreased expression of Akt and GSK-3 and increased IR of p85a PI3K, phosphorylated Akt and GSK-3. In contrast, z-VAD-fmk pretreated cells showed no such changes.

These data show that EGCG affects apoptotic pathway through upstream signal including PI3K/Akt and GSK-3 pathway as well as downstream signal including cytochrome c and caspase-3 pathway. Our findings suggest that activation of PI3K/Akt and inhibition of GSK-3 could be new protective mechanism on pathogenesis of ND.

### 242P. Microglial Activation and Cell Death by 3-Nitropropionic Acid (3-NP)

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Metabolic impairment of neurons has been implicated in several nurological disorders, but it is not at present known whether such metabolic impairment has deleterious effects on microglia. In the present study, we examined whether metabolic impairment induced by 3-NP, an irreversible inhibitor of succinate dehydrogenase, affects the function and viability of microglia in vitro and in vivo. Treatment of 3-NP in HMO6 human microglia cell cultures induced the elevation of intracellular calcium concentration and activation of microglia with production of reactive oxygen species (ROS). Exposure of HMO6 cells to 3-NP also induced cell death as indicated by nuclear fragmentation in a dose- and time-dependent manner. Trolox, an antioxidant agent, was effective in reduction of ROS production and cell death caused by 3-NP. Consistent with in vitro findings, intrastriatal injection of 3-NP in adult rats resulted in an increased ROS production from microglia in vivo, which proved by the oxidation of the reduced MitoTracker probe. ROS production induced by 3-NP was inhibited when trolox was coinjected with 3-NP. Caspase-3 immunoreactivity was demonstrated in OX-42+ microglia in the core and penumbra area of the 3-NP-injected striatum. Apoptotic cell death of microglia was also demonstrated by TUNNEL in the 3-NP-induced lesion. The present results indicate that metabolic impairment by 3-NP in the CNS could involve both activation and cell death of microglia and contribute to pathology in neurodegenerative diseases.

### 243P. Neuronal Expression of αB Crystallin in Cerebral Infarction

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αB crystallin (αBC) is one of the heat shock proteins, which are led by stressful conditions. In normal brains, αBC is present in oligodendrocytes and astrocytes, but not in neurons. Neuronal αBC expression in the central nervous system under pathologic conditions has been investigated in several previous studies, most of which dealt with various neurodegenerative diseases. Neuronal expression of αBC has seldom been studied in cerebral infarction (CI), and the frequency of αBC-positive neurons in the various stages of CI is unknown. To investigate this issue, we examined 48 autopsy brains of patients with CI, and found neuronal expression of αBC in 68.8% of the cases of CI. We found 3 types of αBC-positive neurons: normal morphological, convex, and ballooned neurons. Although αBC-positive neurons were present in the every stage of CI, they were more frequent later than 10 days after the onset of CI than within 10 days after the onset. Previous studies indicated that αBC might have a cytoprotective function as a molecular chaperone, so we speculate that αBC is expressed in neurons subjected to ischemic stress, and exerts a cytoprotective effect on the neurons.

## WORKSHOPS

### Muscular Dystrophies of the Sarcolemma and the Adjacent Extracellular Matrix

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Much of the progress in the elucidation of the genetic basis of the muscular dystrophies has focused on molecules associated with the sarcolemma, composed of the plasmamembrane and the basement membrane. Dystrophin, the protein deficient in Duchenne muscular dystrophy is located at the inner aspect of the plasmamembrane and is associated with a heterogeneous complex of proteins that includes the transmembrane sarcoglycan and dystroglycan complexes. Mutations in 4 of the sarcoglycans (α-, β-, γ-, δ-sarcoglycan) in muscle have been associated with autosomal recessive limb-girdle muscular dystrophy (LGMD 2C-F). The functions of the sarcoglycan-complex in muscle are still not clear, but several hypothesis are emerging. Direct mutations in the dystroglycan complex have not yet been described, however, there is recent and compelling evidence that disorders of O-mannose linked glycosylation can affect the postranslational modification of α-dystroglycan leading to disease. Human disorders implicated include a form of limb-girdle muscular dystrophy (LGMD2I) and a form of congenital muscular dystrophy (MDC1C), both caused by mutations in FKRP (fukutin related protein), as well as a group of congenital muscular dystrophies with involvement of the brain, including Fukuyama muscular dystrophy (mutations in Fukutin), muscle eye brain disease (mutations in POMGnT1) and Walker-Warburg syndrome (mutations in POMT1). Dystroglycan is one of major receptors in muscle for laminin-α2, the heavy chain in laminin 2 (merosin). Mutations in LAMA2, the gene coding for laminin-α2, also cause a form of congenital muscular dystrophy (MDC1A), usually without overt central nervous system involvement except for abnormal signal on T2 weighted MR images. In the adjacent extracellular matrix another new group of disorders has emerged, caused by mutations in the three genes coding for collagen VI. Dominant mutations cause the milder phenotype of Bethlem myopathy, while recessive mutation cause the more severe phenotype of Ullrich congenital muscular dystrophy.

### Nuclear Envelope Proteins and Respective Muscular Dystrophies

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Emery-Dreifuss Muscular dystrophy (EDMD) is characterized by early contractures of Achilles tendons, elbows and spine, together with slowly progressive humero-peroneal muscle wasting and weakness and by adulthood cardiac conduction and/or rhythm defects that evolve to dilated cardiomyopathy and sudden death. We identified in 1999 the first mutation in *LMNA* gene encoding 2 intermediate filaments of the nuclear envelope, lamins A and C, to be responsible of the autosomal dominant form of EDMD. Since this first identification, the role of nuclear envelope components has become more widely recognized with the implication of *LMNA* in 6 other diseases that affect specifically either the skeletal and/or cardiac muscle, the adipose tissue, the peripheral nervous tissue, the bone tissue or more recently premature ageing, leading to the concept of laminopathies. Up to now, more than 136 different *LMNA* mutations were report in 550 individuals. The first study of phenotype/genotype relationships revealed no relation between the phenotype, the type and/or the localization of the mutation, except for the globular tail domain of lamins A/C. Indeed, the resolution of its 3D-structure reveals a relation between the position of the mutated residues and their clinical phenotypes. Studies of the consequence of *LMNA* mutations in the skin cultured fibroblasts from the patients reveal variability in the abnormalities observed, with dysmorphic nuclei exhibiting abnormal pattern of expression of B-type lamins and emerin. Knock-In mice were born recently and will give insights on the pathophysiological mechanisms of *LMNA* mutations.

### Immunohistochemistry in Diagnostic Myopathology

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Immunohistochemistry, in parallel with the rapid expansion of molecular techniques, has revolutionised the diagnosis of neuromuscular disorders. Antibodies to a wide variety of proteins are available but it is essential to do adequate controls, establish baselines and to take account of developmental regulation. Muscle is the tissue of choice for most studies but other tissues in which the protein is expressed, such as skin, can be used. Primary gene defects in recessive conditions are easily observed when there is an absence of a protein, for example dystrophin, emerin, or laminin α2. A reduction of protein can also be detected but this has to be distinguished from a secondary change. Secondary alterations have an important diagnostic role and can direct molecular analysis, for example utrophin, nNOS, and the sarcoglycans. Recent research also suggests alterations in the immunolabelling of α-dystroglycan may be a useful secondary indicator of some forms of CMD. Primary alterations in protein expression in dominant conditions are often difficult to detect with immunohistochemistry but secondary changes, such as reduced sarcolemmal labelling of laminin α1, can be valuable. Other proteins of diagnostic value are foetal myosin, a marker for regeneration and muscle damage, and major histocompatibility class I antigens which are essential for assessment of inflammatory myopathies, as sarcolemmal labelling occurs in the absence of any apparent cellular infiltrate.

### Transmembrane Lysosomal and Enzyme Lysosomal Proteins and Their Roles in Neuromuscular Disorders

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Lysosomes have morphologically unremarkable structures in normal muscles. Nevertheless, lysosomes have important physiological roles in the skeletal muscle since a number of muscle diseases are accompanied by structurally abnormal lysosomes.

Among these disorders, only 2 have been associated with primary lysosomal protein defects, acid maltase deficiency and Danon disease. Acid maltase deficiency, or glycogen storage disease type II, has been well characterized clinically, pathologically, biochemically, and genetically. The disease can be classified clinically into three forms; infantile, childhood and adulthood forms; based upon the severity of the disease. This disease is expected to become treatable in the near future.

Danon disease was originally reported as "lysosomal glycogen storage disease with normal acid maltase" since clinicopathological features apparently resemble those of acid maltase deficiency. However, Danon disease is not a glycogen storage disease since the disease is due to the primary deficiency of a lysosomal membrane protein, lysosome-associated membrane protein-2 (LAMP-2), instead of a glycolytic enzyme. In fact, glycogen is not constantly accumulated and a detailed pathological feature is different from that of acid maltase deficiency. In Danon disease, intracytoplasmic vacuoles are surrounded by membranes that express virtually all sarcolemmal proteins and acetylcholinesterase. This unique pathological feature is also seen in other autophagic vacuolar myopathies, including X-linked myopathy with excessive autophagy (XMEA), and delineates these diseases from other lysosomal myopathies.

### Immunohistochemical Spectrum of Relevant Proteins in Inflammatory and Other Immunomediated Neuromuscular Disorders

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Inflammatory myopathies (IM) are acquired muscle diseases with inflammatory infiltrates at muscle biopsy. Although immune cells may invade dystrophic muscles and might play a role in progression of dystrophinopathies, muscular dystrophies are excluded from this definition. IM mainly include dermatomyositis (DM), polymyositis (PM), and inclusion-body myositis (IBM), and retain their clinical and histopathologic features regardless of whether they occur separately (idiopathic) or in conection with other systemic diseases (eg, HIV infection). DM myopathology reflects a primary involvement of microcirculation mediated by humoral processes with secondary ischaemic changes. Early capillary deposition of the complement C5b-9 membranolytic attack complex (MAC) and perifascicular reexpression of MHC class 1 antigens with or without atrophy are typically observed. PM and IBM are CD8 T-cell-mediated and MHC1-restricted autoimmune myopathies in which muscle cells die from perforin-mediated necrosis. In PM, abnormal MHC1 expression is diffuse and may be detected even in the absence of endomysial inflammatory cells. In IBM, it is multifocal and associated with rimmed vacuoles. A variety of chemokines are released by muscle cells and inflammatory cells, and participate to the recruitement of both deleterious and supportive circulating cells (eg, monocyte/macrophages that support muscle regeneration).

## PLATFORM PRESENTATIONS

### 244. Ultrastructural Approach to Molecularly Defined Dysferlinopathies

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Dysferlin is localized to muscle fiber surface: its functions and the mechanism by which dysferlin-gene mutations allow muscle fiber injury have not been elucidated.

Previous studies of expression profiling in dysferlinopathies showed up-regulation of genes involved in inflammatory-response, proteolysis and protein biosynthesis, and under-expression of some giant sarcomeric proteins related to histopathological features of inflammatory response, degeneration and regeneration. We examined at ultrastructural level 10 muscle biopsies from patients in whom dysferlinopathy was previously molecularly diagnosed (by immuno histochemistry, Western blot and DNA molecular assay) to define the relationship between molecular pathogenetic mechanisms and submicroscopical alterations. In all dysferlin-deficient patients non-necrotic muscle fibers showed: multilayered basal lamina, amorphous-fibrillary material underlying the sarcolemma (regenerative-reparative changes), multiple electrondense globules embedded in the basal lamina, mild loss of peripheral myofibrils, some rods, lipid vacuoles and subsarcolemmal aggregates of mitochondria (degenerative changes), and lymphocytes associated to cell membrane (inflammatory response). Sarcolemmal papillary protrusions both randomly and parallel oriented were seen in most cases, while crowded vesicles and dilated Golgi cisternae were collected in the cytoplasmic side of sarcolemma. The ultrastructural features observed in non-necrotic fibers confirm the association between inflammatory, degenerative and regenerative changes in the pathogenetic mechanism of dysferlinopathies allowing us to consider such alterations as an early diagnostic marker.

### 245. The Myotonic Dystrophy Type 2 (DM2) Protein ZNF9 Localizes to the Sarcomeric I-Band in Normal and DM2 Muscle Fibers.

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*Background*. The genetic defect underlying DM2 is a CCTG expansion in intron 1 of the zinc finger protein 9 (ZNF9) gene in chromosome 3q21. ZNF9 is a 19kDa protein expressed in various tissues, but its cellular localization and function are still unclear. Methods. We applied Western blot (WB) analysis, immunofluorescence (IF), confocal microscopy, and immuno-electron microscopy (IEM) to study the protein expression an the subcellular localization of ZNF9 in normal human and rat skeletal muscle, and in muscle biopsies from DM2 patients. Results. In normal muscle, WB analysis showed ZNF9 as a single band of about 19 kDa MW, with a similar abundance in muscles of different type. IF reactivity for ZNF9 appeared as regular cross-striation bands about 0.9 to 1.1 mm thick in myofibers. In double IF experiments observed by confocal microscopy, ZNF9 showed partial co-localization with proteins localized to the I band or the I-Z band junction such as the sarcoplasmic reticulum Ca/Mg ATPase, the T12 epitope of titin and desmin. Moreover, IEM with immuno-gold techniques confirmed ZNF9 localization to the I-band. Finally, in DM2 muscles the ZNF9 intracellular distribution was comparable to that of control. Conclusions. These data indicate that ZNF9 is abundantly expressed in normal myofibers, where it localizes to sarcomeric I-band, implying a possible association of ZNF9 with contractile or cytoskeletal proteins. The finding that subcellular distribution of ZNF9 is apparently not disrupted in DM2 may corroborate the view that haploinsufficiency is not an issue in the pathogenesis of DM2.

### 246. Late-Onset Plectin Deficient Muscular Dystrophy with Relatively Mild Epidermolysis Bullosa Simplex

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*Introduction*. Plectin is a cytoskeletal intermediate-filament linking protein with a pivotal role in mechanical stress-bearing in skin and muscle. Mutations in the plectin gene are associated either with isolated epidermolysis bullosa simplex (EBS-Ogna type) or with EBS in combination with muscular dystrophy as described by De Weerdt in 1972. Detailed myopathology on the latter was published by Banwell in 1999.

*Clinical Features*. A 40-year-old female presented with an 8-year history of progressive generalised upper limb weakness. Mild EBS was diagnosed at 2 years of age. There was no relevant family history or consanguinity. She had bilateral ptosis, an extra-ocular gaze palsy and mild facial and neck weakness. Generalised upper limb weakness was present with normal axial and lower limb strength. A 1 cm-diameter area of discolouration and a dystrophic fingernail were the only skin abnormalities.

*Pathology*. Chronic myopathic changes were present in deltoid and biceps but not quadriceps. All 3 muscles showed frequent COX negative fibers and multiple cores. On electron microscopy there were occasional enlarged pleomorphic mitochondria, minor Z-band streaming and no nemaline rods. Plectin was absent on immunostaining and Western blotting.

*Conclusions*. We describe a case of plectin deficiency with an unusual phenotype with only mild skin involvement. This diagnosis must be considered in the setting of a late-onset limb-girdle myopathy with external ophthalmoplegia and mitochondrial abnormalities on muscle biopsy.

### 247. In Vitro and In Vivo Magnetic Resonance Spectroscopy in Myopathic Disorders

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*Objectives*. There has been tremendous development in the molecular genetics of muscle diseases but studies on muscle metabolism are limited especially on proton MRS. Hence, this

study was undertaken to: *i)* study muscle metabolism in these cases using in vitro and in vivo proton MRS, and *ii)* to correlate MRS findings with clinical and histological diagnosis. The aim was to establish whether proton MRS has any role in the diagnosis of different types of myopathies.

Methods. All muscle biopsies were snap frozen in isopentane, precooled in liquid nitrogen, serial cryostat sections were cut and examined using H&E, enzyme histochemical and immunohistochemical stains. In 41 muscle biopsies of myopathic disorders, where material was sufficient, in vitro proton MRS was done. For control 9 cases of normal quadriceps from orthopedic OT were taken. In 32 cases, in vivo proton MRS was also done. The metabolites analyzed in in vitro MRS included: *i)* metabolites of carbohydrate metabolism—lactate and glucose; *ii)* metabolites of protein metabolism—creatine, alanine, GLX; *iii)* metabolites of fatty acid metabolism—acetate; and *iv)* membrane metabolites—choline and glycerophosphoryl choline. In case of in vivo MRS water fat ratios were estimated as well as choline creatine ratio.

*Results*. Of the 41 cases, 29 were histologically diagnosed as muscular dystrophies of different types (DMD, BMD, FSHMD, LGMD & EDMD) and 5 as mitochondrial myopathy. In 7 cases, the clinical diagnosis was mitochondrial myopathy but no abnormality was found in the muscle biopsy on routine light microscopic examination as well as enzyme histochemical and immunohistochemical stains.

No statistically significant difference in any of the metabolites was seen in control versus dystrophy cases. However, significant difference were noted between: control versus mitochondrial myopathy, control versus dystrophy, as well as, dystrophy versus mitochondrial myopathy. All metabolite levels were low in dystrophy cases. Mitochondrial myopathy had significantly higher levels of practically all metabolites as compared to dystrophy. No difference in metabolite levels was observed between the different types of dystrophy. The most interesting finding pertain to the 7 biopsies obtained from patients with clinical diagnosis of mitochondrial myopathy, but wherein muscle biopsy was within normal histological limits. All the metabolite levels in these cases were considerably higher than in control and dystrophy cases. However, their metabolite levels were similar to and matched best with those of mitochondrial myopathy. This therefore suggests that in such cases of mitochondrial myopathy, which are not detectable by morphology and wherein facilities for mitochondrial DNA genetics studies are not available, MRS may be a good tool for detecting their metabolite abnormalities which in turn may help in their diagnosis. Low water fat ratio was observed in dystrophy cases as compared to control with 66.7% of cases having a ratio below 1. The most interesting finding was the absence of choline-creatine peak in 66.7% (8/12) dystrophy cases in contrast to 14 cases of control, all of which showed a peak; 62.5% of these dystrophy cases without peak showed fat and fibrosis in muscle biopsy.

*Conclusion*. In vitro and in vivo proton MRS was found to be a good technique to supplement morphology in the diagnosis of myopathies especially mitochondrial myopathies. In vitro proton MRS may possibly be a good tool to differentiate diagnostically difficult cases of mitochondrial myopathy (clinically suspicious but with normal muscle biopsy and no facility for genetic studies) from other types of myopathy, especially dystrophy—increased lactate (more than 20 mMol/L) along with increased alanine and GPC levels will favour mitochondrial myopathy. In vivo proton MRS will possibly be a good non-invasive technique to differentiate muscular dystrophy from normal muscle.

### 248. Phosphorylase Expression in McArdle's Sheep Following Notexin Injections and in Recumbent Animals

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*Background*. McArdle's disease is an inherited myopathy caused by a deficiency of myophosphorylase. Sheep with the disease are being used to study gene therapy regimes and upregulation of phosphorylase.

*Methods*. One hundred microliters of a 5 μg/ml solution of notexin was injected into the semitendinosus muscles of 20 affected sheep, 16 aged from 1 to 6 days and 4 from 2 to 3 years. Biopsies were taken 10, 30, and 60 days after the injections. Muscle samples were also taken from 2 mature affected sheep were recumbent for 11 and 16 days. Frozen sections from the muscle samples were stained with H&E and for phosphorylase activity.

*Results*. Myophosphorylase-positive fibers were seen in areas of muscle damage and regeneration in all the 10 day biopsies from the 16 lambs and 4 mature sheep. Similarly stained fibers were seen in biopsies taken after 30 and 60 days. However the number of positive fibers diminished with time.

Myophosphorylase-positive fibers were also seen in muscles of the recumbent, uninjected, mature sheep. The number of positive fibers varied from muscle to muscle but were most frequent in the extra ocular and extensor carpi radialis, whereas none were seen in the masseter muscles.

*Conclusions*. Regeneration occurs in muscles of sheep with McArdle's disease and it is accompanied by upregulation of phosphorylase.

## POSTERS

### 249P. In Situ Detection of Hepatitis C Virus (HCV) RNA Within Skeletal Muscle Fibers in Patients with Inflammatory Myopathy Associated with HCV Infection

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*Background*. Extrahepatic involvements of Hepatitis C Virus (HCV) have been recently reported. Inflammatory myopathy is one of the manifestations in patients with HCV infection. However, since HCV has not been identified to date in skeletal muscle fibers from those patients, the pathophysiology remains to be clarified.

*Methods*. We examined skeletal muscle biopsy specimens from 4 patients with positive serum HCV-RNA. All patients showed slowly progressive proximal muscle weakness, especially in lower limbs. Real-time polymerase chain reaction (PCR) and in situ hybridization (ISH) for HCV-RNA were performed.

*Results*. PCR study showed the existence of HCV-RNA in muscle specimens from 2 of these patients and ISH positive signals were observed in muscle fibers from the same patients. ISH signal-positive muscle fibers occupied about 5% of all the fibers. Most of these positive fibers appeared atrophic. Positive ISH signals were diffusely

distributed in most of the ISH positive fibers. In some positive fibers, they were observed in subsarcolemmal regions.

*Conclusion*. Our results indicate that HCV might play a direct role in injuring muscle fibers in some cases of inflammatory myopathy related to chronic HCV infection.

### 250P. Mechanism of Skeletal Muscle Apoptosis in Steroid-Induced Myopathy

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In the previous experimental studies, we demonstrated apoptosis of skeletal muscle on steroid-induced myopathy in rats by immunocytochemistry, ISEL, electron microscopy, and DNA electrophoresis (*Neuropathol Appl Neurobiol* 27:396-402; *J Kor Med Sci* 16:467-74). The apoptotic cell death is mediated by Fas antigen expression without involvement of p53 protein. In the present study, we investigated the mechanism with underlying signals of skeletal muscle apoptosis in steroid-induced myopathy of rats. Semi-quantiative changes of apoptosis-related proteins; caspase 8, Bcl-2, Bcl-xL, Akt, p-Akt, Bax, Bad, Bid, and Caspase 9, were determined by Western blot analyses. The levels of caspase 8, Bax, Bad and Bid were higher in steroid-induced myopathy group than control group, while anti-apoptotic proteins, such as Bcl-2, Bcl-xL, were not changed between two groups. Akt protein was not changed, however, p-Akt was slightly decreased. Caspase 9 activity was slightly increased. These overall processes of apoptosis might be involved in skeletal muscle myopathies induced by prolonged treatment of steroids. The apoptotic process presented in this study might be different from classic apoptotic process through glucocorticoid-receptor complex, and then DNA binding in nucleus with subsequent altered gene expression.

### 251P. Clinico-pathologic Studies in Fasciitis: Diffuse Fasciitis with Eosinophilia and Macrophagic Myofasciitis

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*Objective*. Diffuse fasciitis with eosinophilia (DFE) and macrophagic myofasciitis (MMF) are very rare diseases involved in the fascia of skeletal muscle. We investigated clinico-pathologic studies in thesediseases.

*Patients and methods*. Two cases of DFE and 2 of MMF were performed muscle biopsy. Muscle specimens were stained routine histochemical procedures and immunohistochemical methods.

*Results. 1) DFE. Case 1*. A 67-year-old man had a spontaneous pain of shoulder and upper arms and flexion contracture of elbows and knees. His subcutaneous tissues in the upper and lower extremities, shoulders, and back are firm and thickened. *Case 2*. A 52-year-old woman had firm subcutaneous tissue in the lower extremities. In both patients, muscle biopsy showed thickened fascia extending from the subcutaneous tissue to muscle but no inflammatory changes were detected in muscle fibers. *2) MMF. Case 1*. A 50-year-old woman had slowly progressive weakness of upper and lower extremities before one year. *Case 2*. A 27-year-old woman, who suffered from rheumatoid artheritis, had myalgia and firm subcutaneous tissue in the trunk and four limbs. In both patients, muscle biopsy showed inflammatory infiltrates contained macrophages in muscle and fascia.

*Discussion*. In DFE biopsy disclose thickened fascia extending from the subcutaneous tissue to muscle. Whereas in MMF, muscle biopsy shows macrophagic infiltration of the fascia and endomysium.

### 252P. Nemaline Myopathy in 2 Patients Associated with Novel Mutations in the Skeletal Muscle Alpha-Actin Gene (ACTA1)

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Nemaline myopathy is a rare disorder characterized by muscle weakness and excessive formation of nemaline rods in muscle fibers. Nemaline myopathy has been associated with mutations in 5 different genes: ACTA1 (alpha-actin), TPM3 (alpha-tropomyosin slow), TPM2 (beta-tropomyosin), NEB (nebulin) and TNNT1 (troponin T1), which all encode for protein components of the sarcomeric thin filaments.

Mutation analysis of the human skeletal muscle alpha-actin gene (ACTA1) in 7 patients with nemaline myopathy revealed 2 previously not described de novo missense mutations in two patients. Case 1 was a male patient who after birth suffered from pronounced muscle weakness and hypotonia. He had feeding difficulties and required a gastrostomy tube. He failed to achieve any motor milestones. At age 16 years he required 24-hour ventilatory support and had swallowing difficulties. He could not lift his arms against gravity, but he could use his hands to control his electric wheelchair. The muscle biopsy showed nemaline rods in all muscle fibers. He had a missense mutation, G268D, in ACTA1, which was not present in any of the parents.

Case 2 was a female patient who at birth had trouble with poor sucking and general muscle weakness. Two weeks after birth she was hospitalized because of weight loss and she showed signs of hypotonia. She achieved normal motor milestones. At 10 years of age she walked and moved unhindered and could run fairly well. The muscle biopsy showed numerous nemaline rods in all muscle fibers. She had a missense mutation, K373E, in ACTA1, which was not present in any of the parents.

These 2 patients illustrate the marked variability in the clinical features of nemaline myopathy in spite of similar muscle pathology. The described mutations add to the previously reported mutations in ACTA1 associated with autosomal dominant nemaline myopathy.

### 253P. Dermatomyositis with Inclusion Bodies

Yang F; Barnett M; Shingde M; Pamphlett R; Harper C; Pollard J

*Background*. Dermatomyositis (DM), polymyositis and inclusion body myositis (IBM) are 3 distinct categories of inflammatory myopathy with characteristic clinical, immunopathologic morphologic features. DM, an idiopathic disorder with skin and skeletal muscle involvement is often associated with malignancy and responds to variable immunotherapy. Sporadic IBM, on the other hand, is a disorder of the elderly and is unresponsive to immunosuppressive therapy. Muscle biopsies from patients with both DM and IBM often contain necrotic fibers, cytochrome C oxidase (COX) negative fibers and intracellular infiltrates. Despite their morphological similarities, DM is also characterised by perifascicular atrophy and

marked perivascular infiltrates. On the other hand, the characteristic finding in IBM is the presence of rimmed vacuoles, as well as ragged Cred fibers and COX-negative fibers.

*Methods*. To our knowledge, only a single case with overlapping pathologic features of the 2 conditions has been reported. We report a further patient with typical clinical features of dermatomyositis in addition to fibers containing typical rimmed vacuoles and COX-negative fibers in muscle biopsy.

*Results*. Although IBM and DM are clinically and usually pathologically distinct, this report highlights the occasional concurrence of the 2 diseases. IBM should therefore be considered in cases of acquired inflammatory myopathy which are unresponsive to therapy.

### 254P. Autosomal Dominant Hyaline Body Myopathy

Lach B; Bohlega S; Abu-Amero SN; Meyer BF; Al Said Y; Hodgkinson C; Baruch V; Cupler EJ

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*Background*. Hyaline body myopathy (HBM) is a rare congenital myopathy with unique eosinophilic cytoplasmic inclusions (HB) in type I muscle fibers.

*Material and methods*. We report clinical, morphological, immunohistochemical, and preliminary molecular studies on autosomal dominant, clinically non-progressive and progressive forms of HBM in 9 of 15 children, their mother and maternal grandmother.

*Results*. Muscle biopsies (3 patients) showed HB in all type I fibers; they were positive for ATPase at pH 4.3/4.6 and for heavy chain slow myosin(HCSM); some HB were HCSM-negative. HBs were non-reactive for α-B-crystallin, ubiquitin, tropomyosin, actins, desmin, and components of sarcolemma. Ultrastructurally, HBs were granular and filamentous or amorphous, often with fragments of sarcomeres, and surrounded by zone of sarcomeric disorganization. All biopsies showed "myopathic" changes, necrotic fibers, and ingrowth of adipose tissue. Angulated neurogenic fibers and fiber type grouping were consistently present. There was no correlation between the HB and course of disease. However, progressive cases displayed more severe overall myopathy. Linkage studies pointed to association of HBM with chromosome 14.

*Conclusion*. HBM is chromosome 14-linked myopathy, associated with either myolysis or defective incorporation of HCSM into the cytoskeleton. HB contain some additional not yet identified proteins. Neurogenic factors are also involved in the HBM pathogenesis.

## WORKSHOPS

### Generation of Dopaminergic Neurons from Stem Cells

Arenas E

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Stem cells have been suggested to be an optimal source material for transplantation in the treatment of neurodegenerative diseases for their ability to give rise to all neural lineages and to integrate in the brain upon transplantation. One of the main candidate disorders for stem cell therapies is Parkinson's disease (PD), since most of the symptoms in that disorder are related to the progressive degeneration of a discrete population of dopaminergic neurons in the midbrain. Our work has focused in both understanding the signals that regulate the induction and development of midbrain dopaminergic neurons with the aim of developing stem cell-based therapeutic strategies for PD.

In the past we have achieved a coordinated induction of midbrain dopaminergic neurons (in 80% of the cells in culture) by expressing the nuclear receptor Nurr1 in neural stem cells (NSC) and exposing these cells to soluble factor(s) derived from ventral mesencephalic glial cells isolated at the time of birth of dopaminergic neurons. These results have also been confirmed in different Nurr1 expressing cell types, including mouse embryonic stem cells, mouse cortical or midbrain neuronal progenitors and a Nurr1-over-expressing human forebrain NSCs. In order to identify those glial-derived signals we are performing gene chip experiments and protein purification from pure ventral mesencephalic and cerebral cortex glial cultures, to identify genes and proteins specifically expressed by mesencephalic cells during the induction of dopaminergic neurons. Likewise, we are analyzing genes regulated by Nurr1 in NSCs since Nurr1 confers NSCs responsiveness to the inductive signal/s derived from the astrocytes. In summary, our experiments suggest that induction of a specific neuronal phenotype require the convergence of environmental signals derived from neighbor cells, including glial cells, and a genetic program, including the expression of the nuclear orphan receptor Nurr1. We expect that the identification of signaling components involved in the induction and maintenance of a dopaminergic phenotype in stem/precursor cells, will contribute to the development of cell replacement strategies in the treatment of PD.

### Regulation of Neural Stem Cell Differentiation by bHLH factors Hes1 and Hes5

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Embryogenesis progresses according to the scheduled time course, suggesting that cell differentiation is controlled by some biological clocks. We found that the basic helix-loop-helix (bHLH) repressor Hes1 periodically changes its expression in a two-hour cycle after serum treatment or activation of Notch signaling. Serum treatment or Notch activation increases Hes1 mRNA And Hes1 protein, which in turn represses its own transcription by directly binding to its own promoter (negative feedback). Since both Hes1 mRNA and protein have short half-lives (about 20 minutes), they disappear rapidly when transcription is blocked. Thus, the negative feedback is transient, thereby allowing the next cycle of Hes1 expression. This oscillatory expression of Hes1 is observed in many cell types, such as neuroblastoma cells and neural stem cells, indicating that this oscillator regulates the timing of many biological systems. We found that the related gene Hes5 also exhibits oscillatory expression. To understand the roles of Hes factors in neural development, we examined the developing nervous system of Hes-mutant mice. In the absence of Hes1 and Hes5, neural stem cells are not properly maintained and prematurely differentiate into neurons, resulting in disruption of the morphology of the nervous system. Thus, Hes genes are essential for the normal timing of cell differentiation. Relationship between the timing of differentiation and 2-hour cycle oscillation will be discussed.

### Certain Aspects of Neural Stem Cell Biology May Suit Them for CNS Restoration

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An intriguing phenomenon with possible therapeutic dividends has begun to emerge from our observations of the behavior of neural stem cell (NSC) clones in various mouse models of CNS injury and degeneration. During phases of active neurodegeneration, factors seem to be transiently elaborated to which NSCs may respond by migrating (even long distances) to degenerating regions and differentiating specifically towards replacement of dying neural cells. NSCs may "attempt" to emulate in the brain what hematopoietic stem cells do in the periphery: repopulate and reconstitute ablated regions. These "repair mechanism" may reflect the re-expression of basic developmental principles (particularly during particular temporal "windows" following injury) that may be harnessed for therapeutic ends. In addition, NSCs in their native state (or genetically-engineered) may serve as vehicles for protein delivery and appear capable of simultaneous cell replacement and gene therapy (eg, with factors that might enhance differentiation, neurite outgrowth, connectivity, neuroprotection). When combined with certain synthetic biomaterials, NSCs may be even more effective in "engineering" the damaged CNS towards reconstitution.

### Immortalized Multipotent Human Neural Stem Cells for Cell Replacement Therapy in Neurological Disorders

Kim SU, MD, PhD

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Recent advances in stem cell biology have aroused wide and intense attention by investigators as well as general public because of stem cells' broad applications in basic biomedical research and transplantation therapies. Neural stem cells (NSCs) are self-renewing multipotential cells capable of differentiation into neurons, astrocytes, or oligodendrocytes in the CNS. Stable clonal lines of human neural stem cells (HB1) have been generated from a primary culture of human embryonic telencephalon using a retroviral vector encoding v-myc. These self-renewing established cell lines express ABCG2 and nestin, both cell type specific markers of human neural stem cells, and give rise to neurons and glial cells. When HB1 human NSCs were transduced with tyrosine hydroxylase (TH) and GTP cyclohydrolase (GTP-CH) genes to produce

dopamine and implanted into the brain of rat model of Parkinson disease, there was a marked improvement in amphetamine-induced turning behavior and good survival of implanted NSCs. In 3-nitropropionic acid-induced Huntington disease rat model, proactive implantation of BDNF-overexpressing HB1 human NSCs in striatum blocked neuronal cell death by providing a continuous supply of BDNF. These results indicate that the human NSCs should be a great value as a potential vehicle for cell replacement and gene transfer for the treatment of human neurological disorders. (Supported by KOSEF/ BDRC Ajou University and Canadian Myelin Research Initiative).

## POSTERS

### 255P. Role of Retinoblastoma Family Proteins in the Commitment of Rat Neural Stem Cells

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*Background*. Neural stem cells (NSCs) exist in the developing and adult nervous system of all mammalian organism and maintain the capacity to produce neurons, astrocytes and oligodendrocytes in culture.

The multipotentiality of NSCs might be exploited to replace degenerated cells, either through transplantation or by manipulating endogenous cells. Thus, basic studies aiming to characterise the biology of NSCs are of great interest.

*Methods*. We took advantage of an adenovirus mediated delivery system in order to overexpress genes of the retinoblastoma family in NSCs obtained from the periventricular zone of rat pups brain.

*Results and conclusions*. We observed that cultures of differentiating NSCs show a decreased percentage ($p<0.05$) of neurons when pRb or pRb2/p130 are overexpressed. Moreover there is a higher percentage of oligodendrocytes in cultures that overexpress pRb ($p<0.05$). This effect is even greater if these genes are already overexpressed at the onset of differentiation.

These preliminary results suggest that pRb and pRb2/p130 are critical in the commitment and differentiation of NSCs. Furthermore, their role appears to be restricted to a specific temporal window.

### 256P. Adult Bone Marrow-Derived Stem Cells in Muscle Connective Tissue and Satellite Cell Niches: A Novel Finding with Potential Therapeutic Impact

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Postnatal growth and repair of skeletal muscle mainly result from activation, proliferation and fusion of satellite cells that are committed myogenic precursors residing beneath the muscle fiber basal lamina. Besides satellite cells, multipotent skeletal muscle stem cells, the origin of which is unknown have been recently identified in muscle connective tissue (Tamaki et al, *J Cell Biol* 157:571-577). These cells express stem cell markers and may differentiate into muscle cells, endothelial cells, or adipocytes. The distribution of bone marrow derived cells was investigated 1, 3, and 6 months after transplantation of bone marrow from B6-TgGFP transgenic mice to normal irradiated B6 mice, the cytoplasmic green fluorescent protein (GFP) being used as an unambiguous marker of donor-derived cells in host muscle. Abundant GFP+ mononuclear cells appeared in muscle tissue after transplantation and their number increased with time. GFP+ mononucleated cells were located both inside and outside of the muscle fiber basal lamina. GFP+ cells found in sublaminal satellite cell niches expressed unambiguous satellite cell markers (M-cadherin, Pax7, NCAM), their number increased with time and their myogenicity was assessed by appearance of scattered GFP+ muscle fibers at 3 and 6 months post-transplantation. In addition, GFP+ mononucleated cells expressing the stem cell antigens Sca1 and CD34 were detected in the interstitial connective tissue, usually in the vicinity of large and small vessels. As compared to satellite cells, interstitial bone marrow-derived muscle stem cells were less numerous and increased more slowly with time. We conclude that both stem cell marker-expressing cells found in connective tissue and myogenic precursor cells located in sublaminal niches may be derived from bone-marrow in adulthood. Our findings extend the recent observation that bone marrow-derived cells can replenish the satellite cell niches previously emptied by irradiation (LaBarge and Blau, *Cell* 111:589-601).

## PLATFORM PRESENTATIONS

### 257. tTGase Induces Aggregate Formation in Fibroblasts of Huntington's Disease (HD)

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*Background*. Elucidating the mechanism(s) by which polyQ aggregation exerts cellular toxicity now represents one of the most challenging problems in the field. Green (1993) proposed that the polyglutamine tract is a transglutaminase (tTGase) substrate that, in the presence of an active enzyme, can become cross-linked via an isopeptide to polypeptides containing lysyl groups. Indeed, we have demonstrated that synthetic peptides containing consecutive glutamine residues and the polyglutamine tract within huntingtin (htt) are excellent substrates of tTGase and preferentially incorporate into polymers in vitro (Gentile et al, 1998).

*Methods*. In dermal fibroblasts of heterozygous HD, we examined the effects of all-trans retinoic acid (RA) and calcium ionofore (CI) both on cell tTGase expression and activation and on aggregate and apoptosis induction.

*Results and conclusions*. RA treatment of cultured cells, increases tTGase expression, both in normal and pathological cells. When tTGase is activated the expanded htt fragment is more abundant and insoluble high-Mw aggregates in HD fibroblasts appear. The use of RA and CI significantly contributed to aggregate formation. Besides, we showed an increase of apoptotic nuclei, bax, and bcl-2-expression. Caspase transcript was not detected in control fibroblasts while low levels of caspase 6, 3, were detected in HD fibroblasts. In particular, levels of caspase 3 protein significantly correlated with apoptotic nuclei ($p<0.05$) and with bax expression ($p<0.01$). These data indicate that HD fibroblasts, contemporaneously with aggregate formation, modulate the expression of caspases and that caspase 3 seems to be involved in cell death.

### 258. Neuropathological Correlates of Semantic Dementia

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*Objective*. To report on neuropathological findings in 3 cases of semantic dementia, a syndrome sometimes subsumed under the label frontotemporal dementia.

*Material and methods*. The brains were fixed in 4% buffered formaldehyde, extensively dissected, embedded in paraffin, cut and stained by routine staining techniques (Hematoxylin-Eosin, Bielschowsky, Klüver-Barrera, Congo Red) and by immunohistochemistry (IHC) with antibodies against β-crystallin, α-synuclein, tau-β; A4 protein, ubiquitin, GFAP and chromogranin A.

*Results*. All cases were characterized by focal cortical atrophy of temporal lobe but with asymmetric appearance (left>right) and degeneration in posterior orbito frontal cortex. Moreover neuropathological changes gradually disappeared towards the parieto-occipital border and frontodorsal regions, respectively. The posterior part of the superior temporal gyrus was characteristically preserved. Microscopically, there was severe neuronal loss in superficial layers of the temporal and orbitofrontal cortex with spongiformic vacuolization and gliosis. Furthermore ubiquitin positive,but β-crystallin, α-synuclein, chromogranin A, tau-β; A4-negative neurites andintraneuronal inclusions in the superficial layers and dentate granular cells were present. Neurofibrillary tangles,neuritic plaques,ballooned neurons,Pick and Lewy bodies and argyrophilic inclusions were absent.

*Conclusions*. While the neuropathology of these 3 cases suggests a specific form of ubiqutinopathy, the clinical picture met criteria for semantic dementia. It could be suggested that semantic dementia is a distinct clinical and neuropathological entity.

### 259. Neuroserpin Encephalopathy Associated with the PI12 S52R Mutation

Ghetti B; Takao M; Murrell JR; Benson MD; Yazaki M; Glazier BS; Epperson F; Piccardo P; Davis R; Holohan PD; Lawrence DA; Farlow MR

Indiana University; State University of New York; American Red Cross Holland Laboratories.

*Introduction*. Mutations in the PI12 gene are associated with autosomal dominant neurodegenerative diseases. Four mutations (S49P, S52R, H338R, and G392Q) have been found in 5 families.

*Method*. We studied clinically, pathologically, and biochemically individuals carrying the S52R mutation.

*Results*. The proband and his brother presented with myoclonus, generalized seizures and cognitive abnormalities at 24 years of age. The disease progressed and the patients developed global dementia and died at 43 years of age. Neuropathologic studies in these two patients revealed that neuroserpin accumulates in most gray matter regions of the central nervous system and in neurons of the dorsal root ganglia. Neurocytological studies and immunohistochemistry using anti-neuroserpin antibodies showed that neuroserpin accumulates as round, compact deposits in neuronal perikarya and cell processes, forming bodies that are contained within the cisterns of the endoplasmic reticulum. Neuroserpin bodies are not found in any other organ systems. Biochemical studies of cortical tissue from the proband have shown that neuroserpin bodies contain a major protein with a mass of ca. 50 kD. Amino acid sequence of this protein identified it as neuroserpin with a substitution of arginine for serine at residue 52.

*Conclusions*. These studies have expanded the understanding of neurodegenerative diseases associated with mutations in the PI12 gene. (P30 AG10133).

### 260. Frontal Dementia with Bone Cysts (Nasu-Hakola Disease) is Due to Defects of the DAP12-mediated Signaling Pathway

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*Background, patients and methods*. Nasu-Hakola disease (NHD) is a recessively inherited condition with global distribution, clinically characterized by the unique combination of early-onset dementia and bone cysts. We present recent clinical, neuroimaging, neuropathological, and molecular genetic findings in a series of NHD patients and discuss their impact on our views on the molecular pathogenesis of this intriguing disorder.

*Results and conclusions*. NHD usually debuted with pain in ankles and wrists after strain during the third decade, followed by

fractures, due to cystic lesions in the bones of the extremities. Frontal lobe syndrome and dementia began to develop by 30 years of age, leading to death by 40 years of age. At an early stage, neuroimaging disclosed high bicaudate ratios, basal ganglia calcifications, and increased signal intensities of the white matter on T2-weighted MR images. Autopsy findings included advanced frontally accentuated sclerosing leukoencephalopathy with activation of the microglia and microangiopathy. All patients were homozygous either for a loss-of-function mutation of the DAP12 gene or mutations in a second NHD gene, the DAP12-associated receptor TREM2. The DAP12-mediated signaling pathway, deficient in NHD, activates cells of myeloid lineage and seems to play an important, previously unrecognized role in human brain and bone tissue.

### 261. Distribution of Cerebral Cortical Lesions in Diffuse Neurofibrillary Tangles with Calcification (DNTC): a Clinicopathological Study of 4 Autopsy Cases

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We investigated 4 Japanese cases of autopsy-proven diffuse neurofibrillary tangles with calcification (DNTC) both clinically and pathologically, and examined the distribution of the cerebral cortical lesions using hemisphere specimens. The lesions were classified into 3 categories (slight, moderate, and severe). Severe lesions were present in the temporal lobe of the all 4 cases. Severe lesions in the postcentral gyrus, which is not believed to be "Batrophic center" of DNTC, were present in the two patients. Moderate lesions in the postcentral gyrus were evident in the other 2 cases. We postulate that the distribution of cerebral cortical lesions in DNTC have a much wider speatrum than previously believed. Our date also indicate that the unusual clinical features of DNTC reported before, including parietal signs, are roughly consistent with the topographic distribution of cerebral cortical lesions elucidated in this study.

### 262. Immune-Inflammatory Reaction in Neurodegenerative Diseases

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[4] Department of Neurology, Baylor College of Medicine, Houston, Tex.

*Background*. Recent data support the role of immune-inflammatory reaction in neurodegenerative diseases with selective neuronal destruction: amyotrophic lateral sclerosis (motor neurons), Alzheimer's disease (cholinergic neurons of the basal forebrain), Parkinson's disease (dopaminergic neurons in the substantia nigra).

*Methods*. Immunohistochemical techniques were used to evaluate the immune-inflammatory reaction in paraffin embedded CNS sections from the above mentioned diseases.

*Results*. The activation of microglia cells was noted in selective areas of neurodegeneration. Many of these cells were transferred to macrophages and approximately 5% acquired the shape and expressed antigens characteristic for dendritic antigen presenting cells. The cells bearing dendritic cell markers on their surface tend to take position in proximity to vessels. Few of the perivascular monocytic/macrophage cells express also ICAM on their surface. They could be a limited source of dendritic cells originated from the blood. Especially in ALS spinal cords T helper and suppressor cells could also be detected.

*Conclusion*. The data show that cells with powerful antigen presenting function appear in the area of neurodegeneration giving the possibility of the initiation of a local immune-inlammatory process.

### 263. Autoantibody in Stiff-Person Syndrome in Japan

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*Objective*. Stiff-person syndrome (SPS) is a rare and severe disorder of the central nervous system characterized by progressive rigidity and painful spasms of the body musculature. About 60% of patients with SPS have autoantibodies directed against glutamic acid decarboxylase (GAD). In around 5% of patients, SPS is associated with cancer and they are positive for antibodies directed against amphiphysin I. We examined autoantibodies against gephyrin, amphiphysin, and GAD in 8 patients with SPS and one with PER (progressive encephalomyelitis with rigidity syndrome) in Japan to demonstrate the pathogenesis of SPS/PER.

*Methods*. Protein extraction from rat brain was separated by SDS gel electrophoresis. Western blot analysis was performed using serum samples of 8 patients with SPS and one with PER. Human IgG was detected with ECL methods. Immunohistochemical analysis was also done using rat brain tissue.

*Results*. One SPS patient with mediastinal cancer was revealed anti-gephyrin antibody. No autoantibody against gephyrin was found in other 8 patient with SPS/PER. Two patients with SPS and PER were revealed autoantibody against amphiphysin. One patient with SPS was revealed anti GAD antibody.

*Conclusion*. Anti-gephyrin antibody was detected in one patient out of 9. This result indicated that autoantibodies against gephyrin, which is post-synaptic components of inhibitory synapses, would cause SPS. It also suggests that not only anti GAD, amphiphysin, and gephyrin, but also other autoantibodies take part in SPS/PER. We also discussed the relationship between PER and SPS.

### 264. Anti-Glutamic Acid Decarboxylase Autoantibodies in Neurological Disorders

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Glutamic acid decarboxylase (GAD) is the enzyme that catalyses the production of GABA, a major neurotransmitter of the Central Nervous System.

Antibodies to GAD (GAD-Ab) were first recognised in a patient affected by stiff-person syndrome; subsequently they were reported in a large number of cases with type 1 diabetes and recent-

ly GAD-Ab have been described in a number of patients affected by chronic cerebellar ataxia, drug-resistant epilepsy and myoclonus.

GAD-Ab are usually detected by immunohistochemistry (IHC), radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA).

In this study we compared the clinical and immunological findings of 23 patients positive for GAD-Ab grouped according to the neurological disorder. All these patients were positive by IHC for GAD-Ab. We confirmed the antigen specificity by using commercial RIA and ELISA methods using recombinant GAD65 (rGAD65). Our cases, as already reported in the literature, harboured other autoantibodies and were affected by organ-specific autoimmune diseases.

The association of 2 or more organ-specific autoimmune disorders (clinically evident or latent) is usually known as polyglandular autoimmune disease (PGAD).

From data presented in this study, we can assume that ataxia, epilepsy and myoclonus have to be included as possible neurological manifestations of PGAD.

Consequently we would like to stress the importance of identifying GAD-Ab in neurological patients affected by other autoimmune organ-specific disorders; nevertheless, it is important to check for other autoimmune diseases in GAD-Ab-positive neurological patients.

### 265. Altered Immunophilin Levels in Nervous System Degeneration

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*Background*. Immunophilins (IP) are receptors for immunosuppressive drugs like cyclosporin A, FK506, rapamycin and their non-immunosuppressive analogs. These compounds have neurotrophic effects in a variety of systems. The immunophilin receptors for FK506 and rapamycin belong to the family of FK506-binding proteins (FKBP). FKBP levels in the rat brain are up to 50 times higher than in the immune system. Crush injury of facial or sciatic nerves in rat leads to markedly increased FKBP12 levels in the respective nerve nuclei and this increase parallels nerve regeneration.

*Methods and results*. Using immunohistochemistry and Western blotting we showed for the first time that FKBP12 and FKBP52 are expressed in the human nervous system, especially in the substantia nigra-deep gray matter axis. In Parkinson's disease, dementia with Lewy bodies and Alzheimer's disease, FKBP12 levels increase in neurons situated in areas of pathology. This IP colocalizes with synaptophysin and α-synuclein and is present in neurofibrillary tangles, indicating that it may become a novel marker of pathology.

In an MPTP primate model of Parkinson's disease, changes in brain IP expression follow a similar pattern. FKBP levels correlate with the degree of dopaminergic lesion as revealed by PET imaging.

*Significance*. Immunophilins participate in axonal transport, synaptic vesicle assembly and may play a role in neuroprotection against abnormal protein aggregation, suggesting a potential avenue of therapeutic interventions.

## The Huntingtin Syndromes

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Huntington's disease (HD) is a neurodegenerative disorder characterized by the selective loss of striatal neurons of the brain. The genetic defect at the basis of HD is an expansion of a variable stretch of CAG triplets in the first exon of the HD gene, which encodes a widely expressed 348-kDa cytoplasmic protein named huntingtin (Huntington's Disease Research Collaborative Group, 1993). This confers a deleterious gain-of-function to the protein, which becomes toxic for the striatal neurons. More recently, works performed in cells and mice indicate that loss of normal huntingtin function(s) may as well contribute to the pathology (Rigamonti et al, *J Neurosci* [2000]; Dragatsis et al, *Nat Genet* [2000]; for review, see Cattaneo, *Trends Neurosci* [2001]). In particular, huntingtin was found to have a neuroprotective function for brain neurons.

Next we tested the hypothesis that wild-type huntingtin could exert this function by influencing the production of BDNF, an essential neurotrophic factor produced by cortical neurons and delivered to the striatum. The results obtained have led to the conclusion that normal huntingtin affects positively BDNF gene transcription while mutated huntingtin is unable to do so (Zuccato et al, *Science* [2001]). The underlying molecular mechanism will be discussed.

## Human Prion Diseases: Mechanisms of Heterogeneity

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Contrary to Alzheimer's disease, in which the pathology of the sporadic form is repetitive, prion diseases present a wide spectrum of pathological phenotypes not only in the familial but also in the sporadic form and in the form acquired by infection. The familial form includes at least 4 basic pathological phenotypes: Creutzfeldt-Jakob disease (CJD), fatal familial insomnia disease (FFI), Gerstmann-Sträussler-Scheinker disease (GSS), and mixed phenotypes or phenotypes lacking distinctive features. The sporadic form includes at least 6 subtypes with distinct neuropathology. The forms acquired by infection have a pathological phenotype often indistinguishable from sporadic CJD except for the forms acquired by ingestions of contaminated prions such as kuru and variant CJD.

The major determinants of the phenotypic heterogeneity are the prion protein (PrP) genotype and scrapie PrP type as determined by the size of the scrapie PrP resistant to protease digestion. However, recent studies have brought to light other characteristics of scrapie PrP such as aberrant glycosylation and the presence of protease-resistant fragments of different sizes. These findings add further complexity to phenotypic determination scrapie PrP formation and mechanisms of infectivity. These topics along with challenges posed by human prion disease diagnostics and characterizations will be reviewed. The possibity that other neurodegenerative diseases share these mechanisms will be discussed. Supported by NIH and CDC grants and the Britton Fund.

## Neurodegnerative Tauopathies

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Abundant intraneuronal deposits made of hyperphosphorylated tau protein and extracellular deposits of fibrillar beta-amyloid peptides are the defining neuropathological characteristics of Alzheimer's disease. Prominent filamentous tau inclusions and nerve cell degeneration in the absence of beta-amyloid deposits are also hallmarks of neurodegenerative tauopathies exemplified by progressive supranuclear palsy, corticobasal degeneration, Pick's disease and familial frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). The identification of mutations in the tau gene in FTDP-17 has established that dysfunction of tau protein is sufficient to cause neurodegeneration and dementia. So far, 31 different exonic and intronic mutations have been identified. Emerging findings support the hypothesis that tau gene mutations are pathogenic, because they impair the ability of tau protein to interact with microtubules and other molecules, promote the self-assembly of tau, or perturb tau gene splicing, thereby leading to the formation of biochemically and morphologically distinct tau aggregates. Abundant filaments made of hyperphosphorylated tau protein and non-apoptotic neurodegeneration are observed in transgenic mouse models of FTDP-17, making it possible to test therapeutic approaches.

## Models and Mechanisms of Viral Neuropathology

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Animal models have yielded insights into mechanisms by which infectious agents and/or immune factors may impact the developing or mature central nervous system to effect disturbances ranging from subtle abnormalities in behavior to generalized encephalopathy or focal dysfunction. This paper will begin with a review of recent progress in work with: *i)* 2 established rat models of neonatal and adult Bornavirus infection, *ii)* a mouse model of Pediatric Autoimmune Neuropsychiatric Disease After Streptococcal Infection (PANDAS), wherein peripheral exposure to streptococcus and immune adjuvant results in autoimmunity to CNS, and *iii)* an approach to investigating common pathways by which stress interferes with the normal neurodevelopmental trajectory by using a model wherein pregnant mouse dams are exposed to poly (inosine:cytosine). These models will first be considered in the context of retrospective epidemiological analyses implicating infectious agents in selected neuropsychiatric disorders. Thereafter, programs will be described (Pandora's Box Project, Autism Birth Cohort) that have potential to directly investigate the pathogenesis of human diseases by applying high throughput microbiology, toxicology, and genomics to prospective birth cohorts.

## Axon Guidance in the Developing Xenopus Visual System

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Neurons in the eye send out axons that navigate over long distances and through complex pathways to reach their synaptic targets in the midbrain. This remarkable process of axon navigation is a key step in building functional nerve connections in the brain and is performed by millions of neurons during embryogenesis. A major goal of our research is to understand the cellular and molecular mechanisms that underlie axon guidance in the early visual pathway. Retinal growth cones exhibit fast chemotropic responses (minutes) to guidance factors such as netrin-1 and Sema3A in vitro enabling the mechanisms that underlie steering to be explored. When isolated from their cell bodies, growth cones are able to navigate correctly along the visual pathway in vivo and to respond chemotropically to guidance factors in vitro indicating that the steering mechanisms are driven locally and do not require the soma. Our research indicates that guidance cues can trigger rapid changes in protein levels in retinal growth cones: netrin-1 stimulates both protein synthesis and degradation while semaphorin 3A (Sema3A) and lysophosphatidic acid (LPA) elicit synthesis and degradation respectively. Pharmacological inhibition of protein synthesis and/or proteasome-mediated degradation blocks the fast chemotropic responses of growth cones suggesting that these processes mediate guidance decisions. Recent results show that caspase-3, an apoptotic protease, is rapidly activated by netrin-1 and is required for chemotropic responses. Together these findings suggest that localized control of protein levels in growth cones plays a key role in navigation.

## WORKSHOPS

### Molecular Pathology and Pathogenesis of ALS

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The cellular and molecular pathology of ALS provides insights into possible mechanisms of disease. Neuronal and dendritic atrophy, astrocytosis, microglial activation, accumulation of neurofilaments in proximal axons and perikarya of motor neurones (MNs), and characteristic ubiquitin-immunoreactive inclusions (UBIRI) are probably early features. Although degeneration of motor systems predominates, ALS is a multi-system disorder. In ALS without dementia, cognitive and neuroimaging studies show fronto-temporal abnormalities and quantitative morphometry reveals relatively selective loss of calbindin-28k inter-neurons in motor and extra-motor cortex. There is overlap with fronto-temporal dementias and UBIRI are present in the fronto-temporal cortex, hippocampal dentate granule cells, amygdala, and substantia nigra in fronto-temporal dementia with ALS. In FALS with SOD1 mutations SOD1 is associated with UBIRI in some but not all cases. Neurofilament gene mutations in ALS, $CMT_{1E}$, and Parkinson's disease, mutations in dynactin and dynein in human and mouse models of motor system degeneration, and the occurrence of MN degeneration in multisystem disorders (eg, Guam ALS-PD-Dementia, 17q-linked syndromes) suggest that cytoskeletal abnormalities and altered axonal transport are important in the mechanisms of neuronal death in ALS.

### ALS: Links Between Molecular Genetics and Pathology

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Amyotrophic lateral sclerosis (ALS) is a relentlessly progressive and ultimately fatal degenerative disorder in which neurons in the motor cortex, brain stem and spinal cord bear the brunt of the disease. Symptoms begin in one region but become generalised and death is usually due to respiratory failure. The pathological hallmarks of ALS include ubiquitinated, neurofilamentous and hyaline inclusions in the perikaryon and proximal axons of motor neurones. In 5 to 10% of cases there is a positive family history with an incompletely penetrant autosomal dominant pattern of inheritance. Rarely individuals with ALS may have clinical and pathological features of fronto-temporal dementia. Mutations in Cu/Zn superoxide dismutase (SOD1) are detected in 20% of familial and 3% of sporadic cases. Deletions in the neurofilament heavy chain gene have been reported in 1% of sporadic ALS cases. Other loci for familial ALS genes have been described on Chromosomes X, 16, 18 and 20 and for familial ALS and FTD on chromosome 9. Mutations in ALS2/alsin cause a predominantly upper motor neurone disorder with an onset in childhood. Other juvenile loci have been identified for ALS-like on chromosomes 15 (recessive) and 9 (dominant) have also been reported.

SOD1 is ubiquitously expressed but human mutations cause selective motor neurone death. Mice transgenic for several human SOD1 mutations predictably develop a motor disorder with many of the pathological hallmarks of ALS. This paper will explore the genetic links between sporadic and familial disease and how well the human pathology is mirrored in the transgenic mouse models.

### Mechanisms of Selective Motor Neuron Death in ALS

Cleveland DW

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Amyotrophic lateral sclerosis (ALS) is a late onset neurodegenerative disease characterized by selective killing of motor neurons. The most common form of inherited ALS is caused by dominant mutation in Cu-Zn-superoxide dismutase (SOD1). Analysis of transgenic and gene targeted mice have demonstrated that toxicity arises from an aberrant property(ies) unrelated to dismutase activity and loading of the catalytic copper by the Copper Chaperone for SOD1 (CCS). Disease-causing ALS-linked mutants associate with mitochondria only in affected tissues. Like toxicity, this is independent of the CCS and dismutase activity, but is accompanied by covalent damage to mutant SOD1 bound to the cytoplasmic face or within the intermembrane space. Damage to mitochondria from preferential accumulation of missfolded mutant SOD1s in affected tissues thus provides an explanation for the selectivity of mutant SOD1-mediated toxicity that is independent of copper-loading at the catalytic site. By production of mice comprised of mixtures of normal cells and SOD1 mutant cells, toxicity to motor neurons caused by SOD1 mutants is also shown to be non-cell autonomous, that is, it does not derive from toxicity exclusively within motor neurons. Normal motor neurons exhibit symptoms of ALS pathology transferred from surrounding SOD1 mutant-expressing cells. Most importantly, the cellular environment surrounding motor neurons strongly modifies their survival: neurons expressing levels of SOD1 mutants sufficient to provoke ALS-like disease when expressed systemically live much longer when surrounded by wild type non-neuronal cells. These efforts indicate that stem cell replacement of non-neuronal cells can be an effective ALS therapy.

### ALS: the Application of Proteomics and Microarray Analysis to the Problem of Motor Neurone Degeneration

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Mutations in Cu/Zn superoxide dismutase (SOD1) account for 20% of familial cases of ALS. Despite a decade of research, the precise cellular pathways underlying the selective motor neurone (MN) injury in the presence of mutant SOD1, have not been elucidated. The aims of this programme have been to utilise proteomic and microarray technologies to: *i)* identify the effects of mutant SOD1 on the proteome and transcriptional profile of MN, and *ii)* to gain insight into cellular biochemical pathways altered by mutant SOD1.

The NSC34 MN cell line transfected to express mutant SOD1 (G93A, G37R) compared to control cell lines was employed. For proteomic analysis 2-D protein electrophoresis gels were run from the cytosolic and mitochondrial fractions, significant spot changes identified using computerised analysis (Phoretix) and proteins of interest identified by MALDI-TOF mass spectrometry and data-base matching. For gene expression analysis an Affymetrix GeneChip

oligonucleotide microarray system was used with a chip including representation of 12 000 murine genes and ESTs. Verification of important changes was undertaken using several methods, including functional biochemical assays and comparison with tissue from G93A SOD1 mice and human spinal cord.

Analysis of the MN proteome showed significant alteration in the expression of 8 cytosolic proteins in the presence of mutant SOD1. These proteins play important roles in anti-oxidanr defence, proteasome function and NO metabolism. One third of the 12 000 murine transcripts were expressed in NSC34 cells. Few gene changes were observed in the presence of normal SOD1, but 271 (2.3%) of 12 000 of genes were significantly altered in the presence of mutant SOD1, 71 up-regulated and 200 down-regulated. The pattern of the altered transcriptional profile identified several pathways likely to be important in the toxic effect of mutant SOD1 on MN.

## PLATFORM PRESENTATIONS

### 266. Nature of Cortical Neuronal Degeneration in Motor Neurone Disease.

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*Introduction*. The mechanisms by which selective populations of neurones are targeted for cell death in motor neurone disease (MND) are unknown. One possibility is via excitotoxicity due to disturbances in glutamatergic neurotransmission.

*Material and methods*. We measured the density of GABAergic interneurones immunoreactive (IR) for the calcium-binding proteins: calbindin-D28K (CB), parvalbumin (PV), and calretinin (CR), and of SMI-32-IR pyramidal projection neurones using a quantitative image analysis in the primary motor (PMC), dorsolateral prefrontal, and anterior cingulate cortices from 13 MND and 8 control cases.

*Results*. Significantly reduced densities of CB-IR neurones were observed within layers V ($p=0.003$) and VI ($p=0.001$) in MND compared to controls. The densities of CR- and PV-IR neurones were not significantly different between MND and control cases although there were trends towards reduced CR-IR neuronal density within layers V and VI, and of PV-IR neuronal density within layer VI. In addition, a statistically significant reduction in the density of SMI-32-IR neurones was observed within layer V in MND relative to controls ($p=0.009$).

*Conclusions*. GABAergic and glutamatergic neurones are lost in several cortical areas. It is not clear whether these changes occur in tandem, or whether damage to GABAergic neurones is an early change (as suggested by recent electrophysiological findings), could lead to increased glutamatergic transmission and excitotoxic damage to corticospinal tract motor neurones.

### 267. Amyotrophic Lateral Sclerosis with Dementia: Clinicopathological Spectrum and Problem

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We examined 28 cases with motor neuron disease with frontotemporal degeneration. Twenty-one cases presented the clinicopathological features of amyotrophic lateral sclerosis with dementia. The characteristic neuropathlogical finding of ALS-D is frontotemporal atrophy with mild neuronal loss and gliosis with spongy change in superficial layer. The distribution of lesion is accentuated in temporal tip, parahippocampus, amygdala, frontal lobe and insular lobe. In basal ganglia substantia nigra is most severely affected. The feature of motor neuron involvement is essentially consistent with classical sporadic ALS. Bunina bodies and ubiquitin-positive skein-like inclusion are frequently observed. Ubiquitin-positive, tau and synuclein negative intraneuronal inclusions (UI) are observed in small neurons in temporal tip parahippocampus, amygdala and granular cells of dentate gyrus in 20 cases with ALS-D. One ALS-D case showed ubiquitin-positive dystrophic neurites (UN) dominantly. In addition to ALS-D, UI were also found in 2 cases of primary lateral sclerosis, one case of ALS with severe involvement of precentral gyrus, and 4 cases of atypical ALS with frontotemporal atrophy. Ubiquitin-positive dystrophic neurites (UN) are found in one case of ALS-D and one case with atypical Pick disease without Pick body presenting pyramidal tract degeneration. These findings suggest the close relationship among ALS-D, atypical Pick disease without Pick body, primary lateral sclerosis and frontotemporal dementia. UI and dystrophic neurites are recognized as a neuropathological hallmark of MND with frontotemporal degeneration (motor neuron disease-inclusion dementia).

### 268. Increased PARP Expression in Sporadic Amyotrophic Lateral Sclerosis (sALS)

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*Background*. The evidence for increased oxidative stress and DNA damage in sALS prompted studies to determine if the expression of the DNA repair enzyme, poly (ADP-ribose) polymerase (PARP) is increased in sALS.

*Methods*. PARP expression was examined in different post mortem spinal cord and brain regions including motor cortex, parietal cortex and cerebellum by Western blot and immunohistochemistry.

*Results*. Western analysis demonstrated that PARP-immunoreactivity (PARP-IR) was increased 3-fold in spinal cord tissues of sALS patients compared to non-neurological disease controls. Immunohistochemical examination revealed that PARP-IR was predominantly seen in astrocytes and in macrophages, large motoneurons displayed reduced staining compared to controls. PARP-IR was also significantly increased in the motor cortex from sALS patients compared to age-matched controls determined by Western blot. However PARP-IR was also increased in the parietal cortex, and cerebellum of sALS patients, in regions which are usually clinically unaffected in sALS. By immunohistochemistry PARP staining was prominent in the cortical neurons, in the subcortical white matter glial cells and in macrophages.

*Conclusions*. The data suggest that widespread cellular stress on neuronal and glial cells is present in the CNS of ALS patients and support the role of glial alteration in sALS pathogenesis.

### 269. Relationship Between Mutant SOD1 Aggregation and the Redox System in SOD1-mutated FALS Patients and Transgenic Rats (TgR) Expressing Human Mutant SOD1

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*Background*. Living cells produce reactive oxygen species (ROSs). To protect themselves from those ROSs, the cells have developed both an antioxidant system containing SOD and a redox system including peroxiredoxin2 (Prx2, thioredoxin peroxidase) and glu-

tathione peroxidase (GPx). SOD converts superoxide radicals into H2O2, and then Prx2/GPx converts $H_2O_2$ into $H_2O$ and $O_2$, and directly controls the intracellular content of $H_2O_2$.

*Materials and methods*. To clarify the relationship between mutant SOD1 aggregation and the redox system in SOD1-mutated FALS patients and TgR (H46R and G93A), we investigated immunohistochemical studies using antibodies against SOD1, Prx2 and GPx.

*Results*. In human and rat controls, Prx2 and GPx were localized in neuronal cytoplasm. The colocalization of the 3 proteins of SOD1, Prx2, and GPx in neuronal Lewy-body-like hyaline inclusions (LBHIs) in FALS patients and TgR was evident. The intensity of intracytoplasmic Prx2- and GPx-staining in LBHI-bearing neurons in both diseases was either weak or negative.

*Conclusion*. The coaggregation of Prx2/GPx with SOD1 in LBHIs might lead to the breakdown of both the intracellular control of $H_2O_2$ and the redox system itself, amplifying the mutant SOD1-mediated toxicity in SOD1-mutated FALS and TgR.

## POSTERS

### 270P. Alteration of Rough Endoplasmic Reticulum in the Anterior Horn Cells in Amyotrophic Lateral Sclerosis

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There have been reported central chromatolysis (decrease and fragmentation of rough endoplasmic reticulum (rER)) as the early changes in the anterior horn cells (AHCs), the nuclear volume reduction preceding the cytoplasmic RNA decrease, and transcription activity diminution of the ribosomal RNA gene preceding the nuclear size in sporadic amyotrophic lateral sclerosis (ALS). Based on these findings, morphological alteration of the Nissl substance/rER was examined quantitatively in the AHCs in 12 patients with ALS and 8 control subjects. The number of the Nissl substance, whose diameters were more than 1 μm, was 50 ± 23 (mean ± SD) in each cervical AHC of controls, and 19 ± 19 in ALS. The width of the cistern of the rER was about 22 nm in controls, and 18 to 48 nm, with irregular dilatation, in ALS. The number of the attached ribosomes on the cistern was 6.7 ± 2.5 per 0.36 μm length of the cistern in controls, and decreased to be 5.5 ± 3.3 in ALS (ribosomal detachment). The findings observed in the present study indicate that protein synthesis abnormality occurred in rER in the late phase of the degenerative process in the AHCs in ALS.

### 271P. Facial Nerve Avulsion as an Experimental Model to Evaluate the Effects of Neuroprotective Molecules on Adult Motoneuron Degeneration.

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We have utilized an adult rat facial nerve avulsion model to evaluate the effects of neuroprotective molecules on motoneuron degeneration. The right facial nerves of adult Fischer 344 male rats were avulsed and removed from the stylomastoid foramen, and adenoviral vectors encoding glial cell line-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1), insulin-like growth factor-1 (IGF-1), transforming growth factor-β2 (TGF-β2) and metallothionein-III (MT-III) were injected into the facial canal. Animals avulsed and treated with adenovirus vectors showed intense immunolabeling for virus-encoded factors in lesioned facial motoneurons, indicating adenoviral induction of the factors in these neurons. The treatment with adenovirus encoding GDNF, BDNF, TGF-β2 or MT-III after avulsion significantly prevented the loss of lesioned facial motoneurons, improved choline acetyltransferase (ChAT) immunoreactivity and prevented the induction of nitric oxide synthase activity in these neurons. In separate experiments, animals were orally administered solution of a neuroprotective compound T-588 after avulsion. Both free oral administration and oral tube administration of T-588 improved the survival of injured motoneurons and ameliorated their ChAT immunoreactivity. These results indicate that the gene transfer of GDNF, BDNF, TGFβ2 and MT-III and oral administration of T-588 may prevent the degeneration of motoneurons in adult humans with motoneuron injury and motor neuron diseases.

### 272P. Increased Intracellular Calcium and Motoneuron (MN) Degeneration

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*Background*. Motor nerve terminals from amyotrophic lateral sclerosis (ALS) contain increased calcium. Excitotoxicity, free radical injury and immune/inflammatory process all have been postulated to play a role in the pathomechanism of ALS.

*Methods*. Animal models of motor neuron destruction based on these theories were developed to examine whether calcium is increased also in the perikaryon. Electron microscopic histochemical technique was used to visualize calcium.

*Results*. Excitotoxic injury was induced by injection of homocysteic acid in the lumbar subarachnoid space. Dilations and heavy calcium precipitates in the cisterns of the rough endoplasmic reticulum (RER) and of the Golgi system were noted. Destroyed mitochondria released calcium in the cytoplasm.

Free radical injury was studied in G93 A SOD-1 transgenic mice. Calcium laden vacuoles developed originated from the RER and fragmented Golgi system. Mitochondria were similar as in excitotoxic injury.

Intraperitoneal injection of IgG from ALS patients into mice increased the calcium content in motor axon terminals and calcium precipitated also in the dilated cisterns of RER and Golgi system.

*Conclusion*. The raise in intracellular calcium is a common feature in all three forms of induced MN injury and in human ALS. It can activate a cascade of harmful metabolic events leading to cell death.

### 273P. Expression of an Inorganic Phosphate/Vesicular Glutamate Transporter (BNPI/VGLUT1) in the Medulla Oblongata and Spinal Cord of Amyotrophic Lateral Sclerosis (ALS)

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*Background*. VGLUT1 is known to exist in synaptic vesicles of axon terminals and play a presyanptic role in glutamatergic transmission in brain. Although glutamete neurotoxicity has been implicated in ALS, expression of VGLUT1 has not been studied in ALS.

*Methods*. The expression of VGLUT1 was studied in the medulla oblongata and spinal cord of ALS using immunohistochemistry. VGLUT1 was used as a marker of upper motor neuron and expression of VGLUT was analysed in axon terminals to hypoglossal nucleus and anterior horn of spinal cord. The numbers of neurons in hypoglossal nucleus and the anterior horn were used as markers of the severity of lower motor neuronal loss.

*Results*. In hypoglossal nucleus, there is a correlation between the expression of VGLUT1 and the severity of motor neuronal loss. In the anterior horn with severe pyramidal tract degeneration, the expression of VGLUT1 was decreased independently of the severity of neuronal loss. In the anterior horn with mild pyramidal tract degeneration, there is a correlation between the expression of VGLUT1 and the severity of neuronal loss.

*Conclusions*. In the medulla oblongata and spinal cord of ALS, not only upper motor neuronal degeneration but also lower mortor neuronal degeneration cause reduction of VGLUT1.

### 274P. Deficient GLUR2 Editing But Not Expression Causes Neuronal Death of Spinal Motoneurons in ALS

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One of the most plausible hypothesis for sporadic ALS is an exaggerated Ca2+ influx through AMPA receptors. Ca2+ permeability of the AMPA receptor ion channel depends strongly on the edited GluR2 subunit whose pre-mRNA is edited post-transcriptionally at the Q/R site and deficient GluR2 RNA editing at the Q/R site per se causes neuronal death in mice. We analyzed *i)* the expression level of mRNA of each AMPA receptor subunit in motor neurons, and *ii)* the editing efficiency of GluR2 mRNA at the Q/R site in the single neuron level in controls and ALS cases. Spinal motoneurons express less GluR2 mRNA compared to other neuronal subsets. There was no significant difference as to both the AMPA receptor subunits and proportion of GluR2B mRNA to total GluRs mRNA between normal and ALS cases. GluR2 mRNA editing efficiency was significantly decreased in the ventral gray of ALS spinal cord and motoneurons of ALS, but remained 100% in disease and normal controls, including the dorsal gray and white matter of ALS. The present results imply that deficient GluR2 mRNA editing rather than a low relative abundance of GluR2 is highly relevant to ALS etiology.

### 275P. Expression of Ubiquitin-Binding Protein P62 in Ubiquitin-Immunoreactive Intraneuronal Inclusions in Amyotrophic Lateral Sclerosis with Dementia

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*Background*. Amyotrophic lateral sclerosis with dementia (ALSD), corresponding to motor neuron disease type frontotemporal dementia, is neuropathologically characterized by depletion of the motor neurons, degeneration of the extra-motor cerebral cortices and formation of ubiquitin-immunoreactive (not argyrophilic, tau-negative, alpha-synuclein-negative) intraneuronal inclusions. Recently, immunoreactivity of ubiquitin-binding protein p62 in several ubiquitin-containing inclusions (eg, neurofibrillary tangle, Pick body, Lewy body, glial cytoplasmic inclusion) in various neurodegenerative diseases has been reported. We examined the immunoreactivity of p62 in ubiquitin-immunoreactive intraneuronal inclusions in ALSD.

*Subjects and methods*. The subjects comprise 3 males and 2 females of clinically and neuropathologically verified sporadic ALSD. The mean age of onset was 54.2 ± 12.1 (40-74) years. The mean duration of illness was 56.4 ± 43.3 (10-137) months. Formalin-fixed paraffin-embedded adjacent sections from the area including the hippocampus were stained with anti-ubiquitin and anti-p62 antibodies using the streptavidin-biotin method.

*Results*. Ubiquitin-immunoreactive intracytoplasmic inclusions were seen in the granule cells of the hippocampal dentate gyrus in all cases. The proportion of p62-immunoreactive inclusions relative to the total number of ubiquitin-immunoreactive inclusions (p62/Ub ratio) was 30.3 ± 21.9 % (3.1-64.5). There was no correlation between p62/Ub ratio and severity of dementia or duration of illness.

*Conclusions*. Although the main constituent of ubiquitin-immunoreactive inclusions is still unknown, p62 might contribute to the aggregation of ubiquitinated protein in the formation of inclusions in ALSD.

### 276P. Zinc Distribution in the Spinal Cord of an ALS Mouse Model

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*Background*. Both decreases and increases in zinc have been implicated in the pathogenesis of amyotrophic lateral sclerosis (ALS). Mutant superoxide dismutase 1 (SOD1) appears to have a reduced affinity for zinc, suggesting that released zinc may act as a toxic agent to the motor neuron. We therefore examined the distribution of zinc in transgenic mutant SOD1 mice, a model for ALS.

*Methods*. Frozen sections of spinal cord from 12 mutant G93A SOD1 mice and 12 wild-type controls were stained for free zinc with the histochemical technique of autometallography, after intraperitoneal injection with sodium selenite. Mice were studied from the age of 3 months onward.

*Results*. The oldest 5 mutant SOD1 mice had developed weakness by the time of sacrifice. In all mutant SOD1 mice, zinc granules in the spinal anterior horn surrounded motor neuron cell bodies and their processes. The same distribution of zinc was seen in

wildtype mice. The onset of weakness in the mutant SOD1 mice did not alter the zinc distribution.

*Conclusions*. Large changes in the tissue distribution of free zinc do not appear to play a role in the pathogenesis of mutant SOD1 ALS. The possibility, however, remains that zinc-free SOD1, rather than zinc itself, could act as a toxic agent in this disorder.

### 277P. An Autopsy Case of Frontotemporal Presenile Dementia with Motor Neuron Disease and Extramotor Inclusions

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Frontotemporal dementia (FTD) with motor neuron disease (MND) and exramotor inclusions (I) is increasingly being recognized as a form of ALS type-FTD (or a distinct form of ALS ). These have been the subject of recent studies, because of their possible relationship to neuronal degeneration and to dementia of the patients. A 50-year-old man developed dementia about a year before motor weakness of the upper limbs. His MRI showed frontal atrophy. Despite slight muscle weakness of the lower limbs, he had severe dysphagia and died of aspiration pneumonia. His total course was about 2.5 years. The brain weighing 1180 g showed frontotemporal atrophy and no atrophy of the motor cortex. Neuronal loss and spongiosis and many small neurons bearing ubiquitin-positive I (UPI) were seen in the superficial layers (II-III) of the atrophied cortex. Some of Betz cells showed central chromatolysis. In the subcortical white matter and internal capsule there were a number of UPI-bearing oligodendrocyte(oligo)-like cells with conspicuous gliosis. The pyramidal tracts, Goll's fasciculi and anterior horn cells were degenerated. A Bunina body, UP skein and/or spherical I were present. The presence of UPI indicates degeneration of the neurons and the oligo-like cells. Thus, the atrophied gray matter and gliotic white matter should be the primary sites of degeneration, and UPI substance may play an important role in the pathogenesis of this disease.

### 278P. Primary Lateral Sclerosis: An Upper-Motor-Predominant Form of ALS Accompanying Frontotemporal Atrophy and Ubiquitinated Neuronal Inclusions

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We report the autopsy findings of an 82-year-old woman who exhibited upper motor neuron signs in the absence of lower motor neuron signs, which were followed by progressive dementia and frontotemporal atrophy, and died 7 years and 4 months after the onset. At autopsy, the upper motor neuron system was severely degenerated, but the lower motor neurons and innervated skeletal muscles were well preserved. A few lower motor neurons were found to contained Bunina bodies and ubiqutinated skeins in their cytoplasm. However, fragmentation of the Golgi apparatus was not evident in the anterior horn cells examined. Therefore, it was considered that the lower motor neurons were also involved, but the rate of degeneration of these neurons was very slow in the disease process. Frontotemporal lobar degeneration characterized by microvacuolation and ubiquitinated neuronal inclusions and dystrophic neurites in the cortical layer II was also shown. Similar ubiquitinated structures were also observed in the neostriatum. Finally, a survey of the literature based on this patient's clinical and pathological features led us to conclude that a rare clinical syndrome of primary lateral sclerosis is often a rare upper-motor-predominant form of amyotrophic lateral sclerosis that is often accompanied by frontotemporal lobar degeneration with ubiquitinated neuronal inclusions.

### 279P. Motor Neuron Syndrome and Inclusion Body Myopathy Reports: By Chance or Pathogenic Related Association?

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Inclusion body myopathy and motor neuron disease are progressive disorders in which filament involvement represents one of the main hallmarks. For both diseases the pathogenesis is still unknown. We report here on 2 cases with clinico-pathological overlapping of variable upper and/or lower motor neuron involvement and inclusion body myopathy.

The first case was a 68-year-old man with an 18-month history of progressive trunk and limb weakness, fasciculations at 4 limbs, leg spasticity, dysarthria, dysphagia, urge incontinence and cognitive impairment. Electromyography (EMG) showed neurogenic signs and fasciculations on four limb and tongue muscles. Nerve conduction studies were normal. Motor Evoked Potential (PEM) revealed marked pyramidal tract involvement. Serum creatine kinase, cuprum, ceruloplasmin, iron, antiganglioside antibody titer and exercise blood lactate were normal. Cerebrospinal fluid (CSF) examination showed slight increased level of proteins. Brain magnetic resonance imaging disclosed deposits of paramagnetic material in the basal ganglia bilaterally, associated with involvement of the cortico-spinal tract, while dopaminergic receptorial SPECT showed normal basal ganglia marker binding. Muscle biopsy revealed a neurogenic myopathy with rimmed vacuoles.

The second case was a 64-year-old woman with 20-month history of muscle weakness slowly progressing from upper to lower limbs. Neurological examination showed distal muscle weakness and wasting at the upper limbs, deep tendon hypo-areflexia. EMG showed neurogenic signs and fasciculations at 4 limbs. Nerve conduction studies were normal, but H reflex and F late responses were absent at lower limbs. PEM were normal. Blood antiganglioside antibody titer and thyroid hormones dosage were normal, as was CSF examination. Muscle biopsy revealed a neurogenic myopathy with scattered rimmed vacuoles.

These case reports, although not nosologically defined, confirm the clinical variability of the inclusion body myopathy and at the same time rise questions on the possible mechanisms of neuronal damage in motor neuron syndromes, in particular related to the pathogenic significance of the inclusion figures in this neurodegenerative disorders.

## WORKSHOPS

### Malformations of Cortical Development: Nosology and Classification Scheme

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Malformations of cortical development (MCD) are the most common cause of intractable epilepsy in children. Many of these MCD are also associated with infantile spasms and ultimately, with poor neurological outcome. These lesions occur at select epochs during cortical developmental and may involve focal brain regions or the entire cortex. A recent classification scheme has been proposed for MCD which provides a putative mechanistic context for each MCD including malformations due to abnormal neuronal and glial proliferation or apoptosis, malformations due to abnormal migration, and malformations due to abnormal cortical organization. A fourth group includes MCD for which no clear mechanism has been identified (MCD not otherwise classified). Representative disorders of proliferation include tubers in the Tuberous Sclerosis Complex, cortical dysplasia with balloon cells, hemimegalencephaly, gangliogliomas and select microcephalies while disorders of neuronal migration include lissencephaly syndromes, subcortical band heterotopia, and periventricular nodular heterotopia. Disorders of cortical organization include polymicrogyria, schizencephaly, cortical dysplasia without balloon cells, and microdysgenesis. In the group of MCD without a proposed developmental epoch or mechanisms are included mitochondrial and peroxisomal disorders.

To date, 9 MCD genes have been identified. Inheritance patterns are either autosomal recessive, autosmal dominant, or sex-linked. Single gene defects have not been identified for many MCD and in fact, some MCD may result from environmental or multi-factorial etiologies. A complex interplay between single genes and environmental effects may account for several MCD and for others such as cortical dysplasia without balloon cells or microcephaly.

### Epileptogenic Cortical Dysplasia: Genetic Aspects

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Focal cortical dysplasias (FCD) account for almost 10% of lesions associated with pharmaco-resistant chronic epilepsies in humans. A comprehensive histopathological classification system is, however, difficult to establish. Major obstacles represent the lack of experimental evidence for pathogenetic mechanisms underlying FCD as well as difficulties to reconcile histological hallmarks of architectural or cytoarchitectural abnormalities in surgical specimens of uncertain anatomical orientation. Microscopic distinction of slight abnormalities from normal variations of cortical lamination and its developmental timetable is also difficult to obtain in many instances. Molecular-genetical analysis may become, therefore, an important tool. Experimental studies increasingly identify neurodevelopmentally regulated signaling cascades and related syndromes of cortical malformations. Potential candidate genes include the *reelin*-signaling cascade, which regulates early neuronal migration and formation of cortical architecture. TSC1 (*hamartin*) and TSC2 (*tuberin*), genes associated with tuberous sclerosis and interacting with the insulin signaling pathway will also be of considerable impact. In a recent study, we identified allelic loss and frequent polymorphisms of the TSC1 gene in 66% of surgical specimens obtained from patients with focal cortical dysplasia of Taylor's balloon cell type (FCD IIb according to Palmini and Lüders, 2002). These data suggest pathogenic mechanisms related to that of tuberous sclerosis. The identification of such genetic markers will foster the assessment/review of clinico-pathological classification systems addressing focal cortical dysplasias in epilepsy patients.

### Neuropathology of Epileptogenic Cortical Dysplasia

Thom M

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Cortical dysplasias encountered in patients undergoing epilepsy surgery are likely to represent localised malformations. They fall into two main neuropathological groups, according to the extent of the cytological and architectural cortical disruption.

Focal Cortical Dysplasia of Taylor-type (Cyto-architectural Dysplasia) is often visible on MRI. Cortical layering is deranged with normal neurones replaced by cytomegalic, dysplastic neurones and balloon cells. Dysplastic neurones display abnormal somal and dendritic morphology and orientation, highlighted with silver and neurofilament stains. Balloon cells, often more numerous in layer I and the underlying hypomyelinated white matter, show variable immunophenotype and a subgroup of "uncommitted cells" of uncertain neuronal-glial lineage may be identified. Discontinuous regions of dysplasia with intervening normal cortex may be seen. Superimposed chronic inflammation, calcification and gliosis may be present. A reduction in inhibitory interneuronal populations in the vicinity of dysplasia is commonly shown and dysplastic cells may show abnormal expression of immature, developmental and drug resistance proteins which may have implications for their pathogenesis and epileptogenicity.

Microdysgenesis (architectural dysplasia) show more subtle disturbances in cortical lamination with less abnormal cytology and are usually MRI-invisible. An excess of neurones in layer I (including Cajal-Retzius cells), neuronal heterotopia in the white matter, small cortical hamartias and dyslamination characterise this pathology. Similar abnormalities of the cortical architecture may be observed in the lateral temporal lobe in patients with hippocampal sclerosis, including neuronal hypertrophy; the significance of these findings in relation to TLE, the pathogenesis of hippocampal sclerosis and their distinction from normal variations in cortical cytoarchitecture is debated.

### Epileptogenic Cortical Dysplasia: Clinic Pathological Correlations

Spreafico R[1]; Colombo N[2]; Galli C[3]; Tassi L[4]

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*Background*. Malformations of cortical development (MCD) represent a heterogeneous group of focal and diffuse alteration of the cortical mantle resulting from a perturbation of developmental processes. These abnormalities are frequently associated with neurological, cognitive deficits and epilepsy. Since the original obser-

vation of Taylor et al (1971) the focal cortical dysplasia (FCD) are considered, among the MCD, the most numerous epileptogenic forms increasingly revealed by high resolution MRI. However, while some forms of MCD are clearly defined, FCD are variously grouped using disparate terminology Most of the patients with FCD are affected by drug resistant epilepsy and thus candidate for epilepsy surgery.

*Methods*. In a recent retrospective neuropathological revaluation of surgical specimens from epileptic patients operated on for intractable epilepsy we subdivided the FCD into 3 main forms namely: architectural, cytoarchitectural and Taylor type dysplasia. Differences among the histological defined subgroups were confirmed also by immunocytochemical procedures. Subsequently we re-examined the clinical EEG, Stereo-EEG, MRI data and the surgical outcome of the considered patients.

*Results*. These parameters showed that the three category classification based on easily recognized histopathological characteristics, avoiding complicated terminology defines clinically homogeneous groups.

*Conclusions*. These data suggest that a presumptive diagnosis and prognosis could be possible based on electroclinical and imaging data.

## PLATFORM PRESENTATIONS

### 280. Microdysgenesis in Epileptic Brain

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*Introduction*. Cerebral malformations have been occasionally observed in the epileptic brain foci surgically resected from intractable epilepsy patients. Among those, the incidence of so-called "microdysgenesis (MD)" has not been clearly evaluated because diagnostic criteria of MD has been still controversial. Recently, however, peculiar form of MD, characterized by both white matter neurons and perivascular glial satellitosis, was proposed as a definite constitution of MD (Arai et al, *Pathol Internat*, in press), which was focused to clarify its incidence in epilepsy in this study.

*Materials and methods*. Surgical specimens from 532 patients with intractable, as well as control specimens from 22 brains, were studied according to the criteria of MD.

*Results*. On the basis of removed portions, cases could be divided into the following 3 groups; extra-temporal lobe epilepsy group (EXTE group, n = 203), lateral temporal lobe epilepsy group (LTE group, n = 88), mesial and lateral temporal lobe epilepsy group (MLTE group, n = 241). In EXTE group and LTE group, MD was observed in 24 (11.8%) of 203 and 12 (13.6%) of 88, respectively. On the other hand, the incidence of MD was increased in the lateral temporal portions in MLTE group (53/241, 22.0%). No MD was observed in controls.

*Conclusion*. This type of MD constitutes a significant mass among a variety of epileptic lesions, which must be related to epileptogenic mechanism.

### 281. Neuropathology in Surgically Treated Drug Resistant Epilepsy

Galli C[1]; Bramerio E[1]; Citterio A[2]; Meroni A[3]; Lo Russo G[4]; Francione S[4]; Tassi L[4]

[1] Dept. of Pathology, [4]Epilepsy Surgery Centre and [2]Dept. of Neuroradiology, Niguarda Hospital; [3]Dept of Exp. Neurophysiology, Ist. Neurologico "C. Besta," Milano, Italy.

We report the neuropathological findings of 372 consecutive patients (54% male and 46% female) operated on for drug resistant epilepsy at the "C. Munari" Epilepsy Surgery Centre of the Niguarda Hospital in Milan from May 1996 through December 2002. The mean age at surgery was 27 years (SD12) and the mean duration of epilepsy 18 (SD11). Mean age of epilepsy onset was 8 (SD8) and the mean seizure frequency was 40 per month (SD81). MRI investigations prior to surgery was unrevealing in 38 (10%) patients. Surgical procedure was aimed at removing the epileptogenic zone as identified on the basis of the electroclinical and imaging data. The histopathological inspections performed on paraffin sections processed for routine histology and immunocytochemistry revealed that malformative lesions represented the great majority by (52%). Within this group Focal Cortical Dysplasia were 109 (29% of the total population). Tumours were diagnosed in 98 patients (27%) encompassing 37 gangliogliomas and 34 dysembryoplastic neuroepithelial tumours. Degenerative or inflammatory pathologies were the 5% of the total population. Mesial temporal sclerosis frequently associated with cortical dysplasia in the temporal lobe was identifiable in 38 (10%) patients. These data show that cortical dysplasia represent increasingly recognized pathology in drug resistant epileptic patients.

### 282. Seizure-related Secondary Damage of Neocortex in Temporal Lobe Epilepsy (TLE) with Hippocampal Sclerosis (HS): Morphometric Analysis of Cortical Neurons

Sotrel A; Yaylali I; Smith T; Morrison SG; Ragheb J; Melnick S; Duchowny M

Miami Children's Hospital and University of Miami, Fla; University of Massachusetts, Worcester.

Clinical data indicate that surgical intervention in medically intractable partial epilepsy results in a complete control of seizures in 45 to 69% of patients. Surgical removal of the epileptogenic brain tissue and consequent amelioration of frequent uncontrollable seizures may have added long-term benefits, providing that the answer to persistently asked question, "do seizures damage the brain," (*Progress in Brain Research* 2002;135), is an affirmative one. There is already substantial evidence that uncontrolled seizures can induce brain changes in gene expression, cellular morphology & function and neuronal circuitry. We have previously demonstrated a significant neocortical dendritic pathology in patients with partial TLE (*Epilepsia* 35:728). In this study, we performed morphometric analysis of NeuN and CALRET-immunoreactive (ir) neurons and GFAP-ir astrocytes in the seemingly normal neocortex surgically removed from 31 patients with TLE and proven HS. We employed a highly reliable manual neuronal counting technique that we have tested in an earlier study (*Neurology* 41:1117). Patients' age at surgery ranged from 4 to 50 years; epilepsy duration (ED) ranged from 0.5 years to 40 years, averaging at >20 years in 74% of cases. Our findings indicate that there is a highly significant positive correlation between ED and reduction in number of NeuN-ir neurons in cortical layers (CL) II & III (0.0001) and to a lesser extent CL-IV, V & VI (0.002), as well as CALRET-ir neurons in CL-II (0.003). In contrast, clinical history of status epilepticus (3 cases) did not correlate with neuronal loss, nor did patients' age at seizures onset. We believe that the results of our study provide an assenting answer to the above question, and a guidline about the wisdom of early versus delayed surgical intervention in patients with intractable partial epilepsy.

### 283. Role of Insulin Signaling Pathways in Pathogenesis of Tuberous Sclerosis-Associated Tubers and Focal Cortical Dysplasia: Tissue Microarray Analysis

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To evaluate the possible roles of the insulin signaling pathway in the pathogenesis of tuberous sclerosis (TSC)-associated tubers and focal cortical dysplasia (FCD) of Taylor type, immunohistochemical studies were performed on surgically resected corticectomy specimens to detect activated downstream targets of the signaling pathway.

A tissue microarray paraffin block was constructed from blocks of surgically-resected TSC-tubers (n = 8), FCDs with balloon cell (BC) change (n = 22), and cortical dysplasia without BC (n = 9) along with 24 histologically normal neocortices obtained from cases with Rasmussen encephalitis (n = 5), cystic-gliotic encephalopathy (n = 3) and temporal lobe epilepsy (n = 16). Normal-appearing cortex adjacent to dysplastic lesions was also sampled from TSC (n = 4) and FCD (n = 8).

Many abnormal neuroglial cells including dysplastic cytomegalic neurons and BCs were positive for both phospho-S6 and phospho-eIF4G with varying staining intensities in FCD and TSC. Both proteins were less prominently expressed in normal-appearing neocortex ($p<0.05$). The cytoplasmic phospho-p70S6K expression was more specific and most abundant in TSC and much less in other cases including FCD.

These results suggest that constitutive activation of cytoplasmic p70S6K, due to putative dysfunction of either TSC1 or TSC2, may account for the pathogenesis of TSC-tubers, and that FCDs possess a distinct mechanism for activation of the downstream targets (S6 and eIF4G).

## POSTERS

### 284P. Structural Alterations of Principal Neurons and GABAergic Circuitries in the Amygdala in Human Temporal Lobe Epilepsy

Yilmazer-Hanke DM[1]; Faber-Zuschratter HF[1]; Aliashkevichm A[3]; Mundhenk B[2]; Schramm J[3]; Schwegler H[1]; Blümcke I[2]

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Little is known on amygdala pathology in temporal lobe epilepsy (TLE), although this brain region substantially contributes to generation of seizures. In the present study, amygdala pathology was studied in human TLE using cytoarchitectonical, myelin and immunocytochemical stains (glial fibrillary acidic protein, neuronal marker NeuN), and intracellular Lucifer Yellow injections (3-4 controls, 16-17 specimens). Moreover, interneurons containing parvalbumin and GABA decarboxylase were analyzed in the lateral nucleus of amygdala (6 TLE patients, 2 macaca mulatta, 2 Wistar rats). Major histopathological alterations of TLE patients included neuronal cell loss (NeuN: $p<0.05$), fibrillary gliosis, reduction of neuronal soma size ($p<0.01$) and an increase in the maximum spine density of principal neurons ($p<0.01$). There was also a remarkable loss of symmetrical inhibitory synapses on the somata of principal neurons in lateral nucleus of TLE patients that correlated with the extend of perisomatic fibrillary gliosis ($p<0.05$). It was concluded that perisomatic loss of inhibition may lead to a reduced feedback or feed forward inhibition of principal neurons in the lateral nucleus of amygdala. Altogether the structural reoganization patterns of principal neurons and GABAergic circuitries in the amygdala were in a position to induce an enhanced excitability of the amygdala in human TLE.

### 285P. Co-expression of Neuronal and Glial Markers in Focal Cortical Dysplasia: Analysis by Double Labeling Immunofluorescence and Confocal Microscopy

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[2] Brigham and Women's Hospital, Harvard Medical School,Boston, Mass.

*Background*. Focal cortical dysplasia (FCD) is a malformation of unknown etiology in which both the migration and the differentiation of neurons and glia may be abnormal in a restricted region of cortex. Previous studies of FCD have indicated that some cells express markers of both neurons and glia, eg, non-phosphorylated neurofilament and glial fibrillary acidic protein.

*Methods*. The co-expression of neuronal and glial markers was examined by double labeling immunofluorescence and confocal microscopy, in 13 cases from 2 institutions.

*Results*. Three of 4 neuronal markers (neurofilament heavy chain, MAP1b, and MAP2) were co-expressed with glial markers (GFAP and S-100) in balloon cells in most or all of the cases. Interestingly, the staining intensities of these neuronal and glial markers were indirectly related, ie, high levels of neuronal markers were associated with low levels of glial markers, and vice versa. In contrast, NeuN was co-expressed with glial markers in only a few cases.

*Conclusions*. These results are consistent with the idea that focal cortical dysplasia is a disorder of neuronal/glial differentiation, and suggest that there is some phenotypic variation among cases as regards the co-expression of specific markers.

### 286P. Kainate-induced Status Epilepticus and Selective Neuronal Loss in Adult Rat Hippocampus. An Electron-microscopy Study

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*Objective*. It is well known that prolonged seizures induce selective hippocampal neuronal death. Our study was undertaken to elucidate which this death is induced by necrosis or apoptosis.

*Methods*. Prolonged seizures were induced in 8-week-old rats by 12 mg/kg kainate injection. Their hippocampus were examined chronologically by light and electron microscopies.

*Results*. Pyramidal neurons at CA1 and CA3 of hippocampus began dying 3 days and neuronal loss became salient one week after prolonged seizures. Oppositely, TUNEL-positive nuclei were densely scattered at CA1 and CA3, where pyramidal cells were lost. Ultrastructurally, cytoplasm of the affected cell became electron dense and nuclear chromatin condensed partially. Subsequently, nuclear envelope disappeared and the affected cell was fragmentated. However, lysosome and autophagosome were not observed in their cytoplasm.

*Conclusion*. Nuclear debris of pyramidal neurons in our study expressed TUNEL-positive. It is known that necrosis-induced debris also become TUNEL-positive, since nuclear fragmentation is occurred even by necrosis. However, our result indicates that pyramidal cell death caused by kainite induced-seizures results from apoptosis.

### 287P. Ultrastructural Reorganizations and Intracortical Synchronization of the Epileptiform Field Potentials in Intact Cortex at Different Stages of Neuronally Isolated Neocortex Island in Rats

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We investigated correlation between number of boutons and parameters of synchronization of electrical activity in 2 sites in intact right somatosensory cortex in rats at different stages of axonal sprouting elicited by isolation of a cortex slab in left cortex.

With the help of cross-correlation we investigated a temporary delay (parameter of synchronization) in occurrence of epileptiform field potentials in 2 sites located at a distance of 4 mm from each other in intact cortex. Research was carried out in 90 days after full isolation of neural island in symmetrical site in contralateral cortex. Epileptiform activity was induced by penicillin.

Significant increase of number of boutons in III and V layer of intact cortex by 90 days of isolation of neural island in symmetrical site in contralateral cortex corresponded to significant decrease of a delay in electrical activity synchronization. Similar effects were observed in V layer in isolated island 30 days after isolation.

Our results suggest that cortex injury activates formation of new synaptic boutons in contralateral site and increases a degree of synchronization of electrical ectivity, what may influence the epileptogenesis.

Received data suggest, that in rat neocortex the pyramids of III and most probably V layer form a neuronal network which provides cortical synchronization of epileptiform field potentials.

### 288P. The Types of Epileptic Seizures in Neuropsychiatric Lupus Patients

Peterová V[1]; Olejárová M[2]; Bu_ek P[3]; Faber J[4]; Seidl Z[1]; Dane_ J[1]; Dostál C[2]

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The neuropsychiatric lupus (NPSLE) is an autoimmune disorder affecting the brain. Generalized slow wave or epileptic activity in electroencephalography (EEG) and seizures were described in up to 30% of NPSLE patients.

The aim of the study was to evaluate the type of epileptic seizures and the EEG in NPSLE patients.

*The patients and method*. Seventy NPSLE patients were investigated during years 1996-2003. Their average age was 39.1 years. Twenty of them suffered from epileptic seizures.

*Results*. The generalized tonic-clonic seizures were observed in 17 patients. Focal seizures with complex symptomatology were present in 4 patients. One patient sufferred from atonic seizures. Focal seizures with elementary symptomatology were not observed in our patients. Two patients had more types of seizures. EEG abnormality was present in 47 patients.

*Conclusions*. The generalized tonic-clonic seizures significantly prevailed and those patients had predominantly diffuse persistent slow activity in EEG, epileptiform graphoelements were not observed. The long duration of the disease in patients strongly treated by corticoids and cytostatics, high doses of antiepileptics and low disease activity would be the explanation for that.

The study is supported by the research projection CEZ J13/98: 1111.0000. 1 - 206016 of Neurological clinic, 1st Medical faculty Charles university in Prague and the research projection MZ_R 00000023728 of Rheumatologic institute in Prague.

## PLATFORM PRESENTATIONS

### 289. Intravascular Lymphomatosis and Brain Lymphoma: Molecular Genetic Diagnosis Using the Polymerase Chain Reaction on Peripheral Blood Samples

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[5] Department of Pathology, Kaetsu Hospital, Niitsu, Japan.

Peripheral blood samples of the patients were used to examine whether tumor cells were detectable. Thirty cases of suspicious intravascular lymphomatosis (IL) (18 male, 12 female, age 22 to 78) and 12 cases of suspicious brain lymphoma (8 male, 4 female, age 49 to 67) were examined using a semi-nested polymerase chain reaction (PCR) for the immunoglobulin heavy chain (IgH) gene. Primers were set for the IgH variable region framework sequence and J region. Specificity of the amplification was confirmed by cloning and sequencing the amplified product. A monoclonal band was detected in peripheral blood samples from 5 IL cases. The histological diagnosis of IL was confirmed by biopsies or autopsies in 4 cases examined. The sequences obtained from biopsied tissues and blood samples were found to be identical in each case in 3 cases examined. Of the negative cases only 1 was IL. Three of 12 suspicious brain lymphomas were histologically diagnosed as malignant lymphoma, however all blood samples were negative for IgH rearrangement. Thus, this PCR method is likely to be of value in routine diagnosis, especially in IL.

### 290. Expression of Survivin in Primary Cerebral Lymphomas (PCL)

Pal P; Mat A; Broome JC; Rainov NG

*Background*. Survivin is an inhibitor of apoptosis protein that blocks apoptosis by binding to caspases-3 and -7. Its expression has been found to be of poor prognostic significance in several tumor types. This is the first study on expression of survivin in PCL.

*Method*. Immunohistochemistry using monoclonal anti-survivin antibody was performed on formalin-fixed, paraffin-embedded archival tissue from 25 cases of PCL. All these cases were diagnosed as diffuse large B-cell lumphomas. These sections were evaluated for the presence or absence of survivin and its subcellular localisation in tumor cells. The levels of expression were estimated semiquantitatively as absent, low, moderate or high.

*Results*. Results showed that survivin was expressed in nearly 92% (23/25) of samples. Strong cytoplasmic expression was seen in 40% (10/25) and weak staining in 48% (12/25) cases. Nuclear staining was high in 44% (11/25), moderate in 28% (7/25) and low in 20% (5/25) cases.

*Conclusion*. Survivin is expressed in the majority of the PCL cases studied but there is variability in the level of expression. Previous studies have shown that survivin positivity is related to poorer outcome in various malignancies. Our results suggest that variable level of immunohistochemical expression may be employed as a potential prognostic indicator including a possible poor response to treatment.

### 291. A Possible Prognostic Role of Cell Adhesion Molecules Expression in Primary Central Nervous System Lymphomas (PCNSL)

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*Background*. PCNSL are uncommon neoplasms accounting for less then 2% of brain tumors and represent malignant non-Hodgkin’s B-cell lymphomas, which are confined to the CNS.

*Methods*. Looking for some pathological parameters with clinical significance, we analyzed the expression pattern of some adhesion molecules (CD44, CD56, integrins, E-cadherin, beta-catenin) that partecipates in a variety of functions including tumor dissemination and metastasis. We carried out an immunohistochemical study on a pool of patients diagnosed and followed at Istituto Neurologico Besta, sampled for serum and CSF soluble IL-2 receptor (sIL-2R), that seems to be a predictive marker, correlated, in the literature, to serum level of CD44. Out of 23 patients, 15 selected for adequate pathological material, were submitted to immunohistochemistry for cell adhesion molecules.

*Results*. Our data showed that, otherwise other haematological malignancies, CD56 was negative in the PCNSL examined. Integrin and beta-catenin were present only in endothelial cells, without correlations to biological behaviour. E-cadherin was variably expressed in our cases apparently unrelated to the clinical outcome. CD44 was the most diffusely detected in most cases with different degree of expression.

*Conclusion*. As CSF sIL-2R levels were related to the outcome of these patients and sIL-2R seems to be a possible clinical prognostic parameter, CD44 immunostaining arise a possible pathological prognostic parameter in PCNSL.

# SEPTEMBER 18, 2003—HIV NEUROPATHOLOGY IN THE HIGHLY ACTIVE ANTI-RETROVIRAL TREATMENT (HAART)ERA

## WORKSHOPS

### Highly Active Antiretroviral Therapy (HAART) and the Brain

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The advent of HAART has resulted in a major change in the treatment and outlook for those individuals living with HIV infection. However, not all individuals with HIV related cognitive impairments improve while taking HAART, and it is being recognised that a significant number of HAART treated individuals have mild cognitive impairments. Cognitive impairments are more common in intravenous drug users who have far higher rates on HIV encephalitis. The enduring cognitive impairments indicate that not all HAART regimes are effective at treating brain resident virus. While some data is accruing as to which antiretrovirals are present in the cerebrospinal fluid, there is very little information as to which are active in the brain. In this talk I will review which brain changes correlate with HIV related cognitive impairments, the noted differences in brain pathology in different risk groups, the approaches that are available to assess antiretroviral action in brain tissue, and emerging data on which drugs are effective in the brain compartment.

### The Changing Pattern of CNS Pathology

Gray F; Chrétien F; Decouvelaere AV; Scaravilli F

Highly active antiretroviral therapy (HAART) which has been available for most AIDS patients in France since 1996, has resulted in a dramatic improvement of the progression of the disease. From the survey of our series of 343 brains with acquired immunodeficiency syndrome (AIDS) from patients who died between 1985 and 2002, we found both quantitative and qualitative changes in the pattern of human immunodeficiency virus (HIV) neuropathology. Quantitatively, despite a dramatic decrease in the number of autopsies, brain involvement remained a major cause of death. There was an overall decrease in incidence of cerebral toxoplasmosis, cytomegalovirus encephalitis (CMVE) and HIV encephalitis (HIVE) for which successful treatment is available. This contrasted with the unchanged incidence of progressive multifocal leucoencephalopathy (PML) and malignant non-Hodgkin lymphomas (MNHL). However, when looking closer at the three last years, the incidence of diseases affecting patients with severe immunodepression (CMVE, PML, MNHL) decreased in 2000-2002, whereas infections occurring in patients with milder immunodeficiency (toxoplasmosis, varicella-zoster encephalitis [VZVE] or herpes simplex virus encephalitis [HSVE]) became more frequent. In addition, we found uncommon types of brain infection such as BK virus encephalitis or General Paresis. Finally, we described new variants of HIVE: severe leukoencephalopathy with intense perivascular macrophage and lymphocyte infiltration possibly due to an exaggerated response from a newly reconstituted immune system, and also chronic "burnt out" forms of HIVE as VZVE, toxoplasmosis, or PML, possibly associated with prolonged survival, in which neither inflammation nor organisms could be detected.

### Peripheral Neuropathies in HIV-Infected Patients

Authier F-J, MD, PhD

Before the introduction of highly active antiretroviral therapy (HAART), neuromuscular disorders were found in approximately 10 to 20% of patients with HIV-infection, and sub-clinical evidence of peripheral neuropathy could be detected in 50 to 90% of patients with AIDS. HIV-associated peripheral neuropathies classically include inflammatory demyelinating polyneuropathy (IDP), focal peripheral nervous system involvement manifesting by mononeuropathy simplex or multiplex (MM) or polyradiculopathy, and distal sensory polyneuropathy (DSP), a length-dependent axonopathy. The occurrence of the different types of neuropathy is related to the stage of illness and the degree of immunodeficiency. Acute or chronic IDPs tend to occur at the initial stages of the infection and become rarer as immune function deteriorates. Focal neuropathies develop in patients with or without AIDS and may result from various types of vasculitis or Cytomegalovirus (CMV) infection. DSPs constitute by far the most common neuropathy in HIV-infected patients and tend to occur in full blown AIDS. It is currently believed that there is a link between several types of HIV-associated neuropathies and retroviral load. This view has been substantiated in the particular setting of diffuse infiltrative lymphocytosis syndrome (DILS) and may also apply to DSP and HIV-associated ALS-like syndromes.

Introduction of HAART dramatically modified the course and prognosis of HIV infection and resulted in an improved quality of life for the patients. HAART had a significant impact on the epidemiology of HIV-1-associated neuropathies. These treatments have resulted in a marked decrease in the incidence of the peripheral neuropathies related HIV-1, particularly DILS-associated neuropathy, DSP and HIV-1 associated ALS. Likewise, CMV-neuropathy appear to be on the decline. On the other hand, there has been an increase in the prevalence of toxic neuropathies which seems mainly related to antiretroviral nucleoside analogues. Interestingly, neurotoxic complications may be more likely to occur as the survival of HIV-infected individuals becomes longer due to more effective HIV-1 suppression and control of opportunistic infections. Among antiretroviral drugs, only nucleoside-analogue reverse-transcriptase inhibitors (NRTIs) have been associated with peripheral neuropathy. All NRTIs except zidovudine may exert dose-dependent neurotoxic effects, ddC being more toxic than ddI, d4T and 3TC and the combination of ddI+d4T being more toxic than ddI or d4T alone. NRTI-induced neuropathy is clinically virtually undistinguishable from HIV-associated DSP. Temporal relationship between neurotoxic NRTI administration and onset or worsening of DSP, and improvement of clinical and electrophysiological signs of neuropathy after NRTI withdrawal or dose reduction may assess NRTI-induced DSP. The incidence of toxic neuropathies represents a major factor in treatment limitation.

### Brain Pathology of HIV-1 Infected Drug Abusers

Bell JE; Arango JC; Anthony IC; Brannan FW; Brettle RP; Simmonds P

Injecting drug use continues to be, a major risk factor for the transmission of HIV infection. Although the systemic immunosuppressive effects of drug abuse are well known, a basis for interaction with HIV in the central nervous system (CNS) is less well understood.

The Edinburgh cohort of HIV infected drug users displayed a particularly high prevalence of HIV encephalitis prior to the advent or HAART. Although there is some evidence that this is due to infection with a neurovirulent strain of HIV, we have also considered the possibility that host systemic factors, such as drug use, and host genotype influence the outcome. Our studies have shown that opiate abuse activates microglia but does not on its own increase B and T lymphocyte trafficking in the brain. In contrast HIV infection in the early stages upregulates all 3 cell types. While T and B lymphocytes decline markedly in AIDS, microglia are further activated. These contrasting effects are being re-examined in the HAART era.

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Anthony IA, Crawford DH, Bell JE (2003) B lymphocytes in the normal brain: constrasts with HIV-associated lymphoid infiltrates and lymphomas. *Brain* in press.

## PLATFORM PRESENTATIONS

### 292. Brain Tissues from AIDS Patients Contain HIV-1 Transmissible to Human Astrocytes in Culture

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*Background*. HIV-1 replicates in brain in microglial cells and macrophages, but it can also infect astrocytes in vitro and in vivo. Astrocyte infection is either low or non-productive, and it is difficult to detect in vivo.

*Methods*. Eighteen samples of fresh frozen tissue from cortical and subcortical regions of 4 pediatric and 2 adult AIDS autopsies with little, moderate, or severe HIV-1-type lesions were co-cultured with human primary astrocytes, peripheral blood lymphocytes (PBL), or monocyte-derived macrophages (MDM) in vitro. Virus transmission was determined by detection of HIV-1 DNA by PCR in the cell cultures.

*Results*. HIV-1 transmissible to one or more of the cell types tested was present in both cortical and subcortical regions in each case examined. Surprisingly, more samples contained virus that was preferentially infectious to astrocytes than to PBL and MDM in culture. Virus transmission to astrocytes was inhibited by 3′-dideoxycytidine, indicating de novo synthesis of viral products in untreated cells infected by HIV-1.

*Conclusion*. We propose that astrocytes are a readily available target for HIV-1 in the brain and that HIV-1-astrocyte interactions may contribute to the neurological impairment observed in many AIDS patients.

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### 293. New Markers of Neurodegeneration in HIV Encephalitis

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*Rationale*. We hypothesize that in response to highly active anti-retroviral therapy (HAART) we will see a paradigm shift in diagnosis of HIVE. New markers of disease will include: *i)* macrophage infiltration mediated by intrinsic expression of specific integrins, and *ii)* disruption of neuronal axonal protein transport and folding mediated by immunophilins and neurotrophin receptors.

*Study design*. Autopsy brain tissues from 55 HIV positive patients were studied by immunostaining and laser confocal microscopy to assess the abundance and cellular distribution of: macrophage associated integrins, neuronal differentiation and glial activation markers, immunophilins FKBP12 and FKBP52 and the neurotrophin receptor trkB. In selected cases, gene expression was studied by cDNA microarray analysis.

*Results*. We found increased distribution of FKBP12 in the basal ganglia of HIVE cases, predominantly in the axons of dopaminergic neurons. In the majority of the cases we also found an abnormal distribution of the trkB receptors and macrophage integrins. The results of immunohistologic studies were supported by gene microarray analysis.

*Conclusions*. Based on our results, we propose that HIVE in the HAART era will be defined by chronic dysfunction of neuronal axonal transport in the presence of integrin-mediated brain macrophage infiltration. These mechanisms of disease could develop in the absence of a significant systemic or parenchymal viral burden. Finally, in long-term suvirvors we may see an increase in parkinsonian symptoms.

### 294. Virus Tropism Dependent Brain Pathology in Simian Immunodeficiency Virus Infection

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*Background*. Highly active anti-retroviral therapy (HAART) has been successful to reduce progression of Acquired Immunodeficiency Syndrome (AIDS). Nevertheless, recent autopsy analysis of the brain from patients with HIV-1 infection reported same or even increasing numbers of AIDS encephalopathy.

*Materials and methods*. We inoculated macaques with T-cell tropic simian immunodeficiency virus (SIV), SIVmac239, and macrophage-tropic SIVenv/MERT and investigated relationship between lymph node pathology and AIDS related brain pathology.

*Results*. Animals infected with SIVmac239 developed AIDS and showed typical AIDS pathology in the lymph node. The cerebral cortex of these animals showed focal gliosis and degenerative changes in the neuropil. There was no microglial nodules or multinuclear giant cells (MNGCs) in the white mater. Virus infected cells were rarely noted. Animals infected with SIVenv/MERT did not develop AIDS in the same period of infection. The white mater of these animals, however, showed microglial nodules with MNGCs, a pathological hallmark of AIDS encephalopathy. However, no cortical degeneration was observed.

*Conclusions*. Two independent pathogenic processes are suggested in AIDS encephalopathy; AIDS-dependent neuropil degeneration in the cortex, and immune response against invading virus-infected cells in the white matter. Under HAART, the clinical features of AIDS encephalopathy will change to slowly progressive neurological deficits occurring without AIDS.

### 295. Neuropathological Changes in the Human Trigeminal Ganglia in Rabies

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*Background*. Centrifugal spread of rabies virus from the central nervous system to cornea is well known. While the expected route of spread is via the trigeminal ganglia to the cornea, the pathological changes in this ganglion in humans is not well documented. We analyzed the trigeminal ganglia from 23 cases of rabies encephalomyelitis collected at autopsy (paralytic = 22, encephalitic = 1, incubation period: 10 days-4 years) to document pathological changes and attempt viral antigen localization by immunohistochemistry.

*Methods*. Trigeminal ganglia from 23 cases of rabies encephalomyelitis were analyzed by routine stains and immunohistochemistry using polyclonal antibodies to whole rabies virus and nucleocapsid. All cases were confirmed to be rabies by direct immunofluorescence and viral isolation.

*Results and conclusions*. Striking finding was ganglionitis, neuronophagia and degeneration of the ganglion cells with satellite cells proliferation. Efferent nerve roots showed more active axonal degradation than the afferent. Immunohistochemically viral antigen was seen in all cases, within ganglion cells, axons or satellite cells, as diffuse staining of soma or Negri bodies correlating with incubation period. Ultrastructural study in 5, revealed clumps of matrix material and bullet shaped viral particles within neurons and axoplasm. This suggests active participation of this sensory ganglion in spread of the disease and could serve as a reservoir for the virus.

### 296. The Human Neurotropic Polyomavirus JC and its Association with Tumors of the Central Nervous System

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The human polyomavirus JCV is the known etiological infectious agent of the subacute demyelinating disease Progressive Multifocal Leukoencephalpathy (PML), fatal condition frequently seen in patients with AIDS. JC Virus infects more than 70% of the adult population world-wide. Similar to other polyomaviruses, JCV can cause tumors when intracerebrally inoculated into rodents and primates. The tumorogenicity of JC Virus is most likely induced by the viral early gene protein T-antigen, as demonstrated by the occurrence of several neuroectodermal tumors, in T-antigen transgenic animals. Furthermore, T-antigen has the ability to bind and interact with several tumor suppressor proteins, such as p53 and pRb, inducing deregulation of the cell cycle.

We have detected the presence of JC Virus DNA sequences in 69% of a wide variety of human brain tumors, including medulloblastoma, astrocytoma, pilocytic astrocytoma, oligodendroglioma, anaplastic gliomas, glioblastoma multifome, and ependymoma by using 4 sets of primers, that recognize the N- and C- terminal regions of JCV T-antigen, and the Control, VP-1 and Agno regions. More importantly, by immunohistochemistry, we have detected the expression of T-antigen in the nuclei of neoplastic cells in 32.9% of the tested samples.

The results of these experiments provide further evidence on the oncogenic role of JC Virus and its association with human brain tumors. We hypothesize that upon infection of glial cells, completion of the JC viral life cycle results is cytolytic destruction of oligodendrocytes and the development of PML; however activation of the viral early promoter under circumstances adverse to viral replication, can result in the accumulation of T-antigen, transformation and uncontrolled proliferation.

## POSTERS

### 297P. Two Cases of Subacute Sclerosing Panencephalitis in Croatia

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*Introduction*. Subacute sclerosing panencephalitis (SSPE) is rare chronic progressive encephalitis caused by the reactivation of the measles virus or an inappropriate immune response to the measles virus. Following the introduction of vaccination against measles in Croatia in 1968 the incidence of measles decreased rapidly. During the last epidemic in 1998 both children had measles, boy aged of 4 and girl aged 6 months.

*Clinical details*. A 5-year-old girl presented with progressive change of personality and focal grand mal seizures. An 8-year-old boy presented with progressive memory loss, irritability, seizures, involuntary muscle movement and myoclonic jerk. The diagnosis of SSPE was established in both cases by raised measles antibody titers in serum and CSF. Both children died less then 6 months after the first neurological changes. The autopsies were performed.

*Neuropathological findings*. Two pieces of brain tissue were yielded, ranging 10 mm in diameter in both cases. Histologically, perivascular infiltrations of lymphocytes, some plasma cells and macrophages were found. Neuronophagia was common and neurofibrillary degeneration was present occasionally. Intranuclear inclusions bodies were present in both oligodendrocytes and neurons. Ultrastructurally in both cases viral nucleocapsides were seen. Genes of genotype D6 of measles virus were sequenced following RT-PCR amplification from the brain of both children. IFA were also positively stained in tissue samples of both brains.

*Conclusion*. The 2 cases of SSPE reported in Croatia last winter were slow-virus infections of the CNS associated with genotype D6 measles infection in 1998.

### 298P. Experimental Encephalitis by the Vesicular Stomatitis Virus in Mice. Participation of the Glia in the Morphological Alterations of the Brain and Presence of MIP-1α and MIP-1β

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*Introduction*. Experimental models with the vesicular stomatitis virus (VSV), has contributed with important information about the inflammatory response in the resolution of the lesions or in the damage in the central nervous system (CNS). In this study, the evolution of the lesions induced by VSV infection in mice, considering the glial cells, IL-4, MIP-1α and MIP-1β was studied.

*Materials and methods*. C57BL/6 IL-4-/- (knockout for IL-4), C57BL/6 and Swiss mice were employed. VSV Indiana II strain was inoculated through the nostrils. Mice were euthanatized after 2, 4 or 6 days post inoculation. Immunostaining for VSV, astrocytes, microglia, MIP-1α and MIP-1β was performed.

*Results*. VSV caused severe degeneration and necrosis of the neuropil and lesions of neurons. Knockout mice presented more severe lesions. The reactive astrocytosis was intense in all the infected animals, but the density of these cells reduced with the increase of the

gravity of the lesions. Both, the resident and inflammatory cells expressed MIP-1α and in a smaller proportion MIP-1β, in different cellular types (neurons, astrocytes and microglia). On the other hand, the reactive microgliosis was significant in animals with clinical symptoms. The increased density of microglia coincided with the reduction of the number of astrocytes.

*Conclusions*. Possibly the chemokines MIP-1α and MIP-1β have not a decisive role in this phase of the neuroinfection.

### 299P. Expression of Neurotrophic Factors During Central Nervous System Infection of Mice by Vesicular Stomatitis Virus

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*Introduction*. Intranasal inoculation of vesicular stomatitis virus (VSV) has been used as a study model for acute encephalitis. While the mechanisms that initiate and promote leukocyte migration have been studied extensively, the relationship(s) between viral infection, subsequent inflammation and neurotrophic factors production is less well understood. Cronology of expression and tissue distribution of these classes of molecules might determine the ability to regenerate axons in areas of reactive gliosis.

*Materials and methods*. Mouse encephalon supernatant fluid from mice inoculated intracerebrally with VSV strain Indiana 2 was used for intranasal inoculation. Male 25 to 30-day-old C57BI6 mice received $2 \times 10^{3.8}$ units of VSV intranasally on day 0 (Group 1 n = 12). On the same day Group 2 (n = 6) received sterile PBS into nostrils.

*Results and discussion*. Six days after VSV inoculation we observed, besides gliosis, expression of laminin and neural cell adhesion molecule (NCAM) in Group 1. Laminin diffuse immunoreactivity was observed diffusely in the brain and was intense in cerebellar, thalamic and other gray nuclei. NCAN immunoreactivity was also diffuse but less intense and was observed in ependymal cells, neurons in brainstem and in cerebellar cortex. As both molecules are axon outgrowth promoters, they can be involved in the animals' recovery from VSV infection.

### 300P. TGF-β/Smad Pathway Involvement in the Development of Progressive Multifocal Leukoencephalopathy in HIV-1 Infected Patients

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Progressive Multifocal Leukoencephalopathy (PML) is an acute and fatal demyelinating disease of the central nervous system frequently seen in patients with impaired immune systems, particularly AIDS. JCV, a human neurotropic polyomavirus, is the well established etiological infectious agent of this disease. The significantly higher incidence of PML in patients with AIDS than in patients with other immunosuppressive conditions suggests that a direct or indirect interaction between HIV-1 and JCV may be responsible for the activation of the JCV promoter and for the development of PML. Molecular interactions between JCV and HIV-1 via the Tat protein, a potent transcription activator, may be responsible for the direct activation of the JCV promoter. An indirect mechanism through activation of certain cytokines, such as TGF-β and Smads 3 and 4 may also be responsible for the enhancement of JCV gene expression in oligodendrocytes and hence for the developments of PML.

Results from a series of immunohistochemical studies in a collection of 12 well characterized AIDS related PML samples, revealed the presence of the JCV capsid protein in the nuclei of oligodendrocytes and the nuclei and cytoplasm of bizarre reactive astrocytes. HIV proteins, including p24 and Tat were detected in the cytoplasm of astrocytes. Interestingly, Tat, but not p24 was detected in oligodendrocytes, suggesting that extracellular Tat may be taken in and accumulate in oligodendrocytic nuclei, where JCV gene transcription takes place. High levels of TGF-β and its downstream proteins Smad 3 and Smad 4 were detected in JCV infected oligodendrocytes. To confirm these results, in vitro studies demosntrated activation of the JCV promoter by Smad 3 and Smad 4 in glial cells. These observations are consistent with our model suggesting the induction of TGF-β and the stimulation of its downstream factors, including Smad 3 and Smad 4 by HIV-1 Tat, enhancing JCV gene expression and hence contributing to the development of PML in AIDS patients.

### 301P. Experimental Meningoencephalitis by *Encephalitozoon Cuniculi* in Cyclophosphamide-Immunosuppressed Mice

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*Background*. Encephalitozoonosis is an increasingly important opportunistic protozoan infection in immunocompromised individuals, such as HIV-positive patients. This study aims to examine the development of an experimental *E. cuniculi* infection in the central nervous system of mice immunosuppresed with cyclophosphamide (CY).

*Methods*. Adult Balb-C mice were inoculated with $1 \times 10^8$ *E. cuniculi* spores by intraperitoneal route and treated with CY (50 mg/kg, twice a week, intraperitoneal route) during the experimental period. The animals were killed from 15 to 75 days post-inoculation and tissue samples, including brain, liver, kidneys, gut and lungs, were collected and processed for light and transmission electron microscopy investigation. Gram-Chromotrope and Hematoxylin-Eosin staining techniques were performed, as well as GFAP immunohistochemical staining (avidin-biotin method) for astrocyte detection.

*Results*. Multifocal granulomas were seen in all organs and also occurred in the cerebrum, cerebellum and brainstem. An acute lymphocytic, diffuse, non-suppurative menigoencephalitis was observed, with neuronal degeneration and necrosis, demyelination, macrophage infiltration and astrogliosis (with increased GFAP immunoreactivity). *E. cuniculi* spores were seen in the microgranulomas or occurred unassociated with a tissue reaction. The parasites were observed with difficulty in the Hematoxylin-Eosin stained sections. They were Gram-Chromotrope positive, ovoid and measured from 2.4 to $3.2 \times 1.7$ micrometres. Proliferative forms (meronts, sporonts, sporoblasts) and spores were found in parasitophorous vacuoles within neural cells.

*Conclusion*. Experimental encephalitozoonosis in immunosuppressed mice provides an useful model for the study of brain lesions associated with these protozoans in man.

### 302P. Varicella Zoster Virus (VZV) Unusual Neuroligical Complications

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VZV may be associated with number of neurologic complications. Unifocal large vessel infarcts may follow zoster in trigeminal distribution and result from transaxonal transport of virus from trigeminal or cervical afferent fibers innervating vessels. Ophthalmic zoster (HZO) might cause ophthalmoplegic syndromes, rarely associated with secondary optic neuritis. Physiopathological mechanisms are local orbital muscle inflammation, contiguous spread from the fith nerve, associated motor neuropathy or ganglionitis. A 72-year-old man developed paraesthesias in right sided vescicular rash in territory of C5-6-Th6-7 myotomes. Within a week patient developed dysphagia, left facial weakness, hemiparesis, ataxic gait. MRI revealed acute right pontine infarction. Serum contained contained border line IgM antibody to VZV whereas concentration of anti VZV IgG was high. Spinal tap gave normal results. A 66-year-old woman presented right sided painful HZO. One week later she developed homolateral complete external ophthalmoplegia. Serum anti VZV IgG and IgM titers were elevated. CSF contained 23 lymphocytes/$mm^3$, slightly increased protein. On account of abnormal VER, retrobulbar optic neuritis was diagnosed. Orbital CT and MRI scans did not reveal local inflammation. In both cases, in CSF there were no amplifiable VZV DNA. Patients were treated intravenously with acyclovir (10 mg/Kg body weight 3 times daily for 10 days ). Oral steroids were given in the second case This report documents the protean manifestation of VZV infection and reactivation.

### 303P. Varicella Zoster Virus and Trigeminal Ganglia

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Zoster (shingles) is caused by reactivation of varicella zoster virus (VZV) in ganglia. Zoster sine herpete is defined as radicular pain without rash. Here we describe pathological features in an immunocompetent adult who experienced relentless trigeminal zoster sine herpete. A 45-year-old developed progressive numbness, pain and sensory loss in the distribution of the right trigeminal nerve. There was no history of zoster rash. Although brain imaging initially was normal, a mass developed at the base of the right brain, and was removed at craniotomy. Histopathological examination revealed ganglion and nerve with widespread chronic inflammation. There was marked loss of neurons and myelinated fibers. Many cell nuclei was enlarged and vesicular, and some contained Cowdry type A inclusions. Most of the inclusion-bearing cells had elongated nuclei consistent with satellite cells.

Schwann cells or fibro blasts. There was no vasculitis, hemorrhage or hemosiderin deposition, though some vascular walls were thickened. Most of the lymphocytes were CD43+ T-cells; fewer were CD20+ B cells. PCR revealed the presence of VZV DNA, but not CMV DNA. Immunohistochemistry revealed VZV-specific antigen, but not HSV-specific antigen, in cells throughout the ganglion. Overall, the pathological and virological data confirmed that the disease was due to VZV ganglionitis.

### 304P. Pathological and RT-PCR Studies of Canine Distemper Virus Infection in Taiwan

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*Background*. Canine distemper virus (CDV) is a highly contagious viral disease, primarily affecting the dogs and other members of the canine family. The aim of this study is to clarify the role of CDV infection in the dogs suffering from demyelinating encephalitis in Taiwan and to compare pathological examination and RT-PCR in the diagnosis of CDV infection.

*Methods*. Thirty-two clinically CDV suspected dogs were used in the study. All cases were subjected to pathological investigation. Nucleic acid extraction was carried out on frozen tissues and body fluids. The RNA samples were tested for the presence of CDV specific nucleic acid by nested RT-PCR. DNA fragments with expected sizes were purified and sequenced.

*Results*. Fifteen (46.9%) out of 32 cases were diagnosed as CDV on the base of intranuclear/intracytoplasmic eosinophilic inclusion bodies. The occurrence of inclusion bodies in descending order was the CNS, lymph nodes, urinary bladder, lung, alimentary tract and skin. With the aid of RT-PCR assay, 26 cases (81.3%) were diagnosed as CDV positive. Two cases of CDV-inducing demyelinating encephalitis were also found.

*Conclusion*. RT-PCR can raise CDV diagnostic rate and also provides a more rapid, specific, and sensitive method to diagnose CDV infection than pathological observation.

### 305P. Immunohistochemical Detection of Astrocytes and T Lymphocytes in Brain of Brazilian Cattle Naturally Infected with Bovine Herpes Virus Type 5 (BHV-5)

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*Background*. Bovine herpesvirus type 5 (BHV-5) is a neurovirulent alphaherpesvirus that causes fatal meningoencephalitis in cattle. This virus has a high incidence of neurological disease in South American countries, mainly Brazil and Argentina. Based on the fact that the inflammatory response of the brain in viral encephalitis by BHV-5 is not completely well-known, 13 cases of viral meningoencephalitis in Brazilian cattle were subjected to immunohistochemical analysis for inflammatory response description.

*Methods*. All the cases showed severe neurological signs followed by death observed after natural infection with BHV-5. Mild to moderate histological inflammatory changes in the brain and cerebellum characterized the neurological infection that showed meningitis, mononuclear perivascular cuffing, gliosis, hemorrhage, and macrophages (Gitter cells) accompanying great areas of malacia. None of the cases showed intranuclear inclusion bodies, however, in six of them was possible the isolation of the BHV-5. Samples of brain and cerebellum of all animals were analyzed by immunohistochemical staining using polyclonal antibodies against

CD3 to detect T lymphocytes and polyclonal antibodies against glial fibrillary acidic protein (GFAP) to detect astrocytes.

*Results and conclusions*. The results indicate a prominent astrocytic response in different degrees of reactivity, to the time that T lymphocytes constituted a high percentage of the mononuclear cells, which characterize inflammatory response.

## WORKSHOPS

### The Mitochondrial Machinery

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Mitochondria are ubiquitous in eucariotic cells and the site of oxidative phosphorylation. These cytoplasmic organelles, which display an amazing plasticity of distribution and shape appearing as bean-like organelles or extended reticular network, are thought to be arisen about 1.5 billion years ago from a symbiotic association between a glycolytic proto-eucariotic cell and an oxidative bacterium. Relics of this endosymbiotic event are the double membrane structure, the circular genome with specific transcription, translation and protein assembly systems, the presence of specific transmembrane carrier systems for ions, metabolites, and proteins, and the numerous and diverse degradative and biosynthetic reactions carried out in addition to OXPHOS. Also, the complex nature of mitochondrial function implies that specific interactions between the cell and the organelles are established: among these are the nucleo-mitochondrial communications concerning the assembly of the respiratory chain, and the central role of mitochondrion in the control apoptosis.

The aforementioned complex functions of mitochondria can be summarize and described on the basis of the current information available as: *i)* transport systems; *ii)* synthetic metabolic pathways; *iii)* degradative metabolic pathways; *iv)* oxidative phosphorylation; and *v)* control of apoptosis.

Among these functions the most relevant is the fundamental reaction of life, ie, oxygen activation and the conservation of energy in cell respiration (OXPHOS), a reaction carried out by the mitochondrial respiratory chain. Notably, from the genetic standpoint, the respiratory chain is unique as it is formed by means of the complementation of 2 separate genetic systems: the nuclear genome and the mitochondrial genome.

### MtDNA-Encoded Encepalopathies

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The small, maternally inherited mitochondrial DNA (mtDNA) has turned out to be a veritable Pandora's box of pathogenic mutations. Fifteen years into the era of "mitochondrial medicine," over 150 point mutations and countless rearrangements have been associated with a variety of mitochondrial encephalomyopathies. Yet, pathogenesis is only partially explained by the rules of mitochondrial genetics and remains largely uncharted territory. This is especially true for mitochondrial encephalopathies. For instance, it is not apparent why epilepsy, which is almost invariably part of the mitochondrial encephalomyopathy, lactic acidosis, strokelike episodes (MELAS) and of the myoclonus epilepsy ragged red fibers (MERRF) syndromes, is rarely seenin patients with Kearns-Sayre syndrome (KSS). The pathogenesis of the stroke in MELAS is unclear, although both metabolic insult and microvascular dysfunction may play a role. The characteristically high CSF protein concentration in KSS (>100 mg/dl) also remains puzzling. We will try to provide clues to these and other riddles using immunocytochemical and ultrastructural studies of the brain.

### Nuclear Encoded Mitochondrial Encephalomyopathies

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In contrast with the wealth of information gained on mitochondrial DNA (mtDNA) mutations, the number of nuclear oxidative-phosphorylation (OXPHOS)-related genes that have been proven to be associated with mitochondrial syndromes is still rather small. However, progress in this field has been made in the last five years, to such an extent that a clinical-genetic classification can be proposed for these defects, as follows:

*1*. Defects in nuclear genes encoding structural components of OXOHOS complexes.

*2*. Defects in genes encoding assembly factors of OXPHOS complexes.

*3*. Defects in genes altering the stability of mtDNA.

*4*. Defects in genes encoding factors involved in the biogenesis of mitochondria, including OXPHOS.

Thus, several structural genes in complex I are responsible for severe infantile lactic acidosis with neurological deterioration. The same picture can be associated with mutations in the biggest subunit of complex II, while mutations in the remaining three subunits of complex II can cause tumors of the paraganglia or adrenal medulla. Mutations in five assembly genes for cytochrome c oxidase have been linked to Leigh syndrome (SURF1), severe lactic acidosis of infancy (SCO1 and COX10), and early-onset fatal,cardio-encephalo-myopathy (SCO2 and COX15). The gene responsible of a further variant of Leigh syndrome, French-Canadian type, also associated with COX deficiency, was previously mapped to chromosome 2p16-21 and has recently been identified as encoding LRPPRC (leucine-rich PPR-motif containing protein). LRPPRC encodes an mRNA-binding protein likely involved with mtDNA transcript processing, suggesting an additional mechanism of mitochondrial pathophysiology. Mutations in another assembly gene, BCS1L, specific to complex III, were found in severe infantile complex III deficiency syndromes, including GRACILE ((growth retardation, aminoaciduria, cholestasis, iron overload, lactacidosis, and early death). Abnormality of cardiolipin metabolism has been found in Barth syndrome (X-linked mitochondrial myopathy, cardiopathy, neutropenia, short stature, and 3-methyl glutaconic aciduria), and 2 peculiar syndromes have been associated with CoQ10 deficiency in muscle. Mutations in different genes are associated with dominant or recessive syndromes affecting mtDNA abundance or integrity. Finally, several neurodegenerative disorders have been attributed to mutations in mitochondrial proteins indirectly related to respiration and energy production. In spite of these recent discoveries, in many cases the diagnosis of mitochondrial disease is based solely on the detection of a biochemical OXPHOS defect or of typical morphological abnormalities in the muscle biopsy, while the genetic basis of the disorder remains unknown.

## Mitochondrial Involvement in Neurodegenerative Diseases

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A growing body of evidence indicates that mitochondrial dysfunction may play an important role in the pathogenesis of many neurodegenerative disorders. Because mitochondrial metabolism is not only the principal source of high energy intermediates, but also of free radicals, it has been suggested that inherited or acquired mitochondrial defects could be the cause of neuronal degeneration as a consequence of energy defects and oxidative damage. In addition, mitochondria are involved in several apoptotic pathways that result in cell death. Mitochondrial respiratory chain dysfunction has been reported in association with primary mitochondrial DNA abnormalities, and also as a consequence of mutations in nuclear genes directly involved in mitochondrial functions, but defects of oxidative phosphorylation and increased free radical production have also been observed in diseases that are not due to primary mitochondrial abnormalities. In these cases, the mitochondrial dysfunction is likely to be a secondary effect, which, nevertheless, could be of importance in precipitating a cascade of events leading to neuronal cell death. In either case, understanding the role of mitochondria in the pathogenesis of neurodegenerative diseases could be important for the development of therapeutic strategies in these disorders. We will discuss the arguments in favor and against the concept that mitochondrial dysfunction play an important role in the pathogenesis of some of the most common neurodegenerative disorders such as Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, and Huntington's disease, focusing on experimental evidence from the literature and from our own research group.

## Lipid Muscle Disorders: Molecular Update

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Patients with lipid myopathy (LM) are typified by inhibited beta-oxidation and increased intramyocellular lipid content. Most defects pertain to fatty acid transport, ie, carnitine transporter defect or carnitine palmityl transferase 2; other to defective enzymes of beta-oxidation, ie, very longchain acylCoA transferase, trifunctional enzyme, medium and short chain acylCoA transferase. Primary carnitine deficiency is an autosomal recessive disorder characterized by recurrent hypoketotic hypoglycaemia and cardiomyopathy. A gene encoding for the high affinity carnitine transporter OCTN2 is mutated in these patients. Carnitine supplementation is an efficacious treatment and reverses the dilatative cardiomyopathy. Other LM are characterized by muscle weakness, myoglobinuria and exercise intolerance.

Some cases of late onset LM respond to exogenous riboflavin supplementation and are characterized by ethylmalonic adipic aciduria. We explored mechanism(s) of biochemical derangement in these riboflavin responsive myopathies (RRM): complex 1 and 2 and mitochondria respiratory chain are usually decreased and there is defective medium and short chain acylCoA dehydrogenases. Mitochondrial FAD and FMN are also down regulated.

## POSTERS

### 307P. Rare Mitochondrial Abnormalities in the Central Nervous System in Leigh Syndrome

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*Background*. Leigh syndrome is a heterogenous disease connected with mutations in mitochondrial DNA genes responsible for different enzymatic defects. Since in literature there is scarcely of data concerning pathological changes in mitochondria in the central nervous system we examined them in one of the mitochondrial disorders-in Leigh syndrome.

*Material and methods*. We examined brain and spinal cord of 15-year-old girl with 5 years history of progressing symptoms of lower and upper motor neuron involvement, involuntary movements and behavioral changes. In EEG generalized pathological changes were found. EMG showed features of chronic neurogenic damage. The patient died because of respiratory insufficiency.

*Results*. On light microscopy we found symmetrical necrotic foci with macrophage infiltration, microglial reactivity and vessel proliferation in caudate nucleus, brain stem and spinal cord gray matter. Ultrastructural study revealed enlarged mitochondria with vacuolization and decrease in the number of cristae. In some mitochondria large electron dense granules and spiculate structures were seen as well as lipid droplets within mitochondrial matrix.

*Conclusions*. In our case ultrastructural changes in mitochondria were different from the ones described in mitochondrial myopathies. The most intriquing finding was lipid storage in mitochondria.

### 308P. A Novel Mutation in the Mitochondrial tRNA Phenylalanine Gene Associated with Mitochondrial Myopathy and Encephalopathy

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We report a novel heteroplasmic T→C mutation at nt position 582 within the mitochondrial tRNA phenylalanine gene of a 70-year-old women with mitochondrial myopathy and encephalopathy. No other family members were affected, suggesting that our patient was a sporadic case. The muscle showed frequent ragged red fibers and 43% cytochrome c oxidase (COX) deficient fibers. The mutation alters a conserved base pairing in the aminoacyl acceptor stem. The mutation load was 70% in muscle homogenate and varied from 0 to 95% in individual muscle fiber segments. COX-negative fibers showed significantly higher levels of mutated mtDNA (>75%) than COX-positive fibers (<55%). This mutation adds to the previously described four pathogenic mutations in the tRNAPhe gene, indicating that this gene is a hotspot region for mtDNA mutations.

### 309P. Metabolic Muscle Adaptation to Aerobic Training in Mtiochondrial Myopathies

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Mutations of mitochondrial DNA at the skeletal muscle level are responsible for insufficient ATP production and deranged metabolism, a main effect of which is represented by abnormal production of lactate. Aim of this study was to evaluate in 10 patients affected by chronic progressive external ophthalmoplegia (CPEO) and large-scale mtDNA rearrangements functional adaptation of skeletal muscle to supervised constant workload 10-week aerobic training, by assessing modifications of anaerobic lactate threshold and relating it to muscle biopsies parameters.

A significant decrement of exercise lactate (-36.5%, $p<0.01$) and lipoperoxide (-13.7%, $p<0.05$) levels after training was observed. The training-related decrement in exercise peak lactate correlated with cytochrome c oxidase (COX) enzyme activity ( $r=-0.84$, $p<0.05$), the number of COX- ($r=0.75$, $p<0.05$) and ragged red fibers ($r=0.68$, $p=0.05$). On the contrary, no relation was found with the amount of deleted mtDNA in muscle biopsy.

These results indicate that aerobic training can be beneficial also in those CPEO patients more severely affected by mitochondrial dysfunction. The level of COX activity in muscle biopsy rather than the amount of mutated mtDNA seems to be a useful predictor for the effectiveness of aerobic training program, suggesting some gene expression mechanisms in mediating muscle adaptation to training itself in these patients.

### 310P. Myopathy with Trabecular Muscle Fibers: Analysis of Mitochondrial Function by Morphological, Biochemical and Molecular Studies

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Neuromuscular Outpatient Clinic, Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto, USP, Brazil.

*Introduction*. Trabecular or lobulated fibers are skeletal muscle fibers characterized by the presence of subsarcolemmal, more or less triangular areas of increased oxidative enzyme activity on histochemical preparations of muscle biopsies. These morphological findings reflect abnormal distribution of intermyofibrillar mitochondria, and are unspecific abnormalities that can be seen in various proportions in a number of neuromuscular diseases. More recently, a distinct subgroup of patients with a slowly progressive, adult-onset muscle disease was identified, in which trabeculated fibers were the main pathological change in muscle biopsy (Durcan, 1994 resumo congresso Neurology, 1994). Because clinical picture was more or less homogeneous and prevalence of trabecular fibers was high in this group of patients, the definition of the trabecular fiber myopathy as a new clinical entity was proposed (Weller et al, 1999). The pathogenetic mechanisms associated with this disorder remain to be determined.

*Objective*. Since the maldistribution of mitochondria in trabecular fibers may have a deleterious effect that could be the cause of clinical symptoms, we performed an extensive analysis of various aspects of mitochondrial function in a group of five patients with high percentages of trabecular or lobulated fibers in muscle biopsy.

*Patients and methods*. We identified 5 patients followed at the Neuromuscular Outpatient Clinic of Hospital das Clínicas da Fac-

uldade de Medicina de Ribeirão Preto, USP, whose muscle biopsies, collected between 1999 and 2002, presented high proportion (>40%) of trabecular or lobulated muscle fibers. Mitochondrial morphology was evaluated by light and electron microscopy. The panel of histochemical reactions performed included succinate dehydrogenase (SDH) and cytochrome c oxidase (COX). Immunohistochemical studies for the expression of subunits I and IV of COX, and alpha subunit of ATPase were also performed. Biochemical analysis included the activities of complexes I to IV of respiratory chain. Mitochondrial DNA (mtDNA) was analyzed by Southern blot.

*Results*. Four patients presented adult-onset, slowly progressive muscle weakness, predominantly affecting limb girdles. In one of the patients the symptoms began during childhood. Morphological analysis revealed the presence of ragged red fibers in 1 patient and COX negative fibers in 3. Biochemical studies of respiratory chain complexes were normal. Immunohistochemical analysis of the expression of COX subunits I and IV was unremarkable. Studies of the ATPase subunit alpha, however, disclosed reduced expression of this protein in 4 patients. Southern blot analysis of mtDNA showed normal results.

*Conclusion*. Reduced expression of the ATPase alpha subunit in trabecular muscle fibers may reflect a dysfunction of mitochondrial ATPase in those fibers, which would result in insufficient ATP production through oxidative phosphorylation and subsequent muscle weakness.

### 311P. Expression of the Mitochondrial Thioredoxin System in Rat and Human Central Nervous System

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The antioxidant thioredoxin mitochondrial system is composed of thioredoxin reductase 2, thioredoxin 2, peroxiredoxin 3 (PRDX3) and peroxiredoxin 5 (PRDX5). Many neurological disorders are associated with nitrosative stress. Moreover, mitochondria have been implicated in such a process. In Alzheimer's disease and Parkinson's disease, the hippocampus and the substantia nigra, respectively, are the first regions of the brain to develop neuropathological lesions and are, in advanced cases, heavily affected areas. To determine whether the vulnerability of certain neuronal populations could be due to a less efficient protection by the mitochondrial thioredoxin system, we analyzed by immunohistochemistry the expression of the thioredoxin mitochondrial system in the brain of adult rats and normal humans. Our results show that in the hippocampus, this antioxidant system is well expressed in CA4 and CA2/3 neurons as well as in neurons of the subiculum. However, immunoreactivity was weak in neurons of the CA1 and of the dentate gyrus. Dopaminergic neurons were not immunoreactive in rat substantia nigra. Furthermore, we showed that overexpression of human mitochondrial PRDX5 and PRDX3 in CHO cells protects against cytotoxicity induced by nitric oxide and peroxynitrite donors. Altogether, our results suggest that the vulnerability of certain neuronal populations to nitrosative stress could be partly due to a weak expression of the mitochondrial thioredoxin system.

### 312P. HIF-induced and Mitochondrially Impaired Lymphoblast Cell Lines Show Upregulation of Genes in Response to Bioenergetic Crises

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*Background*. Having the long-term goal of developing treatment strategies for patients with impaired mitochondrial respiration, we sought to determine the mechanism(s) by which mitochondrially depleted cells (Rho[0]) survive in the absence of oxidative phosphorylation. Eukaryotic cells sense oxygen and adapt to hypoxic conditions by regulating many genes including hypoxia inducible factor-1 (HIF-1).

In this study, we sought to identify downstream pathways affected by HIF induction that might enable cells to function during bioenergetic crises.

*Methods*. Nuclear extracts were prepared and HIF-1α induction was assessed via Western blotting. Total RNA was isolated from both HIF-induced and Rho(0) cells. Biotinylated, fragmented cRNA for each sample was prepared using the Affymetrix Genechip protocol. Differential gene expression was analysed using the U-133A Affymetrix Genechip oligonucleotide probe arrays and Microarray Suite 5.0.

*Results*. HIF-1α induction was established at the protein level in the Namalwa (Rho+) cell line. Microarray analyses showed an increase in certain HIF-related genes of mitochondrially depleted cell lines. Pathways involved in energy metabolism, cell cycle control and oncogenesis were also upregulated.

*Conclusions*. Microarray studies have allowed us to evaluate differential gene expression in Rho(0) cell lines when compared to cells having normal respiration, thus identifying a possible role for HIF-related genes in cells/tissues with mitochondrial dysfunction.

## PLATFORM PRESENTATIONS

### 313. Retinal Degeneration and Dysplasia (rdd) as a Model of Cell Death and Neurodegeneration

Bridges LR[1]; Burt DW[2]; Morrice DR[2]; Hocking P[2]; Mohamed MD[3]; Thaung C[1]; Inglehearn CF[3]

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[2] Roslin Institute, Midlothian, United Kingdom.

*Introduction*. The retinal degeneration and dysplasia (rdd) mutant in the chicken is a putative model of human retinitis pigmentosa. We have mapped rdd to a region homologous to human chromosomes 9p and 5q and identified 3 candidate genes.

*Methods*. Eyes were fixed in formalin, paraffin-embedded, sectioned at 4 μm and stained with H&E.

*Results*. Six eyes from 6 affected birds (2 each aged 3-4 months, 9-14 months and 4 years) were compared with controls. At all ages the retinal pigment epithelium showed marked attenuation. The photoreceptor and outer nuclear layers were virtually absent and gliotic. The inner nuclear, ganglion cell and other layers were thinned.

*Discussion*. The histological appearances are similar to those previously reported (Randall et al, *Exp Eye Res* 37:337). Linkage mapping places rdd in a region homologous to human chromosomes 9p and 5q. Candidate disease gene loci include PDE6A, WGN1 and USH2C (Burt et al, in press).

*Conclusion*. Retinal degeneration and dysplasia (rdd) in the chicken is a putative homologue of primary photoreceptor cell degeneration in humans and may be valuable as a model of neural cell death and neurodegeneration.

Supported by the Wellcome Trust.

### 314. Pro-apoptotic Bcl-2 Homolog Bax and Bak are Required in TRAIL-induced Apoptosis

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*Introduction*. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induces apoptosis through activation of apoptosis-initiating caspase-8. The caspase-8 cleaves Bid, which in turn interacts with Bcl-2 family proteins to activate mitochondrial pathways. Here, we show that Bcl-2 homolog Bax and Bak regulate mitochondrial release of apoptotic factors in TRAIL-induced apoptosis.

*Results*. Bax and Bak were expressed in glioma cells. To define the roles of Bax and Bak in TRAIL-induced apoptosis, we examined Bax wild (Bax+/-) and Bax-deficient (Bax-/-) cell lines. TRAIL induced cleavage of caspase-8 and bid, mitochondrial release of cytochrome c and Smac and cleavage of downstream caspase-3 and -9 in Bax+/- cells. TRAIL triggered cleavage of caspase-8 and Bid, but neither mitochondrial release of the apoptotic factors nor cleavage of downstream caspases in Bax-/- cells. To overcome the resistance of Bax-/- cells to TRAIL, we treated the cells with cisplatin and camptothecin and showed that this pre-treatment sensitized TRAIL-induced apoptosis through caspase-8-initiated caspase cascade and mitochondrial release of the apoptotic factors. Cisplatin and camptothecin upregulated Bak in Bax-/- cells and transfection of Bak antisense cDNA in Bax-/- cells results in the cell resistance to TRAIL and drugs.

*Conclusion*. The study indicates that Bax is required for TRAIL-induced apoptosis and chemotherapeutic drugs upregulate Bak and thus rescue TRAIL sensitivity in Bax deficient cells.

### 315. Intracytoplasmic Inclusion Bodies in Hippocampal Pyramidal Cells Probably Induced by Anti-Cancer Agents

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National Kikuchi Hospital, Division of Clinical Research, Japan.

*Background*. The inclusion bodies thus far unknown were detected in the cytoplasm of pyramidal cells of the hippocampus in the brain of an autopsy case of a 67-year-old female who died 10 months after the development of mucoepidermoid carcinoma originated from the right parotid gland. The patient was treated with administration of many kinds of anti-cancer agents, such as 45 mg of bleomycin, 2000 mg of endoxan, 2250 mg of 5-FU, and 64 mg of mitomycin C.

*Methods*. Paraffin sections were prepared in an usual manner for lightmicroscopy. Specimens for electron-microcopy were prepared from paraffin blocks of hippocampus and observed with a Hitachi H-7000 electron-microscope.

*Results*. The inclusion bodies were round shaped with the concentric triple layer structure discriminable to have the first layer in the innermost of this body, the second layer in between and the third layer at the outermost, surrounded with hollow. Eosin and Bodian staining exhibited distinguished stainability of the second layer, while less stainability of the first layer and no stainability altogether of the third layer. Electron-microscopically the 3 layers were all consisted of 10 to 20 nm filaments differing in the collective density depending on the layer.

*Conclusions*. It can be assumed that the anti-cancer agents may have affected cytoskeleton formation resulting in the induction of these inclusions.

### 316. Association of SUMO and PML with Ubiquitinated Inclusions

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*Background*. Small ubiquitin-like modifier (SUMO) is a protein moiety that is conjugated to lysine residues in a variety of target proteins. Conjugation of SUMO (SUMOylation) regulates nuclear transport and intracellular localization of the target proteins. It is known that promyelocytic leukemia protein (PML), a SUMOylation substrate, is involved into formation process of ubiquitinated intranuclear inclusions (NIIs) in polyglutamine diseases. We investigated how SUMO and PML are associated with ubiqitinated intranuclear and cytoplasmic inclusions.

*Methods*. Sections from midbrain of Parkinson disease (PD), pons of MSA and medulla oblongata of neuronal intranuclear hyaline inclusion disease (NIHID) were stained with anti-ubiquitin, α-synuclein, SUMO-1, PML antibodies.

*Results*. NIIs in NIHID and Marinesco bodies in PD contained SUMO-1 and PML. PML was found only in small NIIs, as reported in polyglutamine diseases. Unlike PML, SUMO-1 was found also

in large NIIs. GCI, NCI and Lewy bodies contained ubiquitin and α-synuclein, but neither SUMO-1 nor PML. NIIs in NIHID showed similar PML localization with those in polyglutamine diseases, indicating that PML play a similar role in process of NII formation. SUMO and PML were not always co-localized in the NIIs. Other SUMOylated proteins than PML and/or unconjugated SUMO-1 might be involved in the NIIs. SUMO-1 was preferentially included in NIIs, indicating that SUMO mediated nuclear transport might relate to the mechanism how SUMO-1 is involved into NIIs.

## POSTERS

### 317P. Blood Brain-Barrier Breakdown is Associated with Decreased Angiopoietin-1 and Endothelial Apoptosis

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Angiopoietins (Ang) belong to a novel family of endothelial growth factors that function as ligands for the endothelial-specific receptor tyrosine kinase, Tie-2. Ang-1 decreases permeability and has an anti-apoptotic effect on endothelium of noncerebral vessels, whereas Ang-2 can act as a Tie-2 antagonist and block the effects of Ang-1. Our previous studies of the rat cortical cold injury model demonstrated decreased Ang-1 protein in vessels showing BBB breakdown suggesting that this may contribute to increased vascular permeability possibly by endothelial apoptosis. This study was undertaken to determine whether apoptosis occurs during BBB breakdown in this model by TUNEL staining and immunohistochemical detection of activated caspase-3 and BAX proteins over a period of 6 hours to 6 days post injury.

Vascular endothelial cells and neurons in the lesion were positive for activated caspase-3, BAX proteins and TUNEL staining from 6 hrs to day 2 post-injury during the period when marked BBB breakdown was present. TUNEL staining peaked at day 1 post-injury whereas activated caspase-3 and BAX labeling peaked at day 2. Dual labeling for Ang proteins and activated caspase-3 demonstrated that vessels showing endothelial cell apoptosis showed increased Ang-2, but not Ang-1 protein.

This study demonstrates the importance of Ang-1 in maintaining vascular homeostasis in normal brain and supports the hypothesis that loss of normal Ang-1-Tie-2 interaction is associated with endothelial apoptosis and BBB breakdown.

### 318P. TRAIL Inhibits Glioma Growth, But Does Not Cause Hepatotoxicity in Mice with Chimeric Human Livers

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*Introduction*. TRAIL triggers apoptosis in tumor cells but not in most normal cells and may prove to be candidate in cancer therapy. However, tagged forms of recombinant TRAIL has been reported to kill human astrocytes and hepatocytes, raising the concern of safety in clinical usage of TRAIL.

*Results*. Human astrocytes and hepatocytes were freshly isolated from patients. These cells underwent apoptosis, as demonstrated by systematic cleavage of caspase-8, caspase-3 and DNF fragmentation factor 45, after exposure to FasL, but not to non-tagged native sequence human TRAIL (amino acids 114-281). In contrast, the TRAIL induced apoptosis in human glioma and melanoma cells. To further evaluate the TRAIL, we generated SCID/alb-uPA mice with human livers. Repeated injections of the TRAIL in the chimeric mice inhibited tumor growth, while the chimeric livers appeared to be normal morphologically. Mouse serum tests for human a1-anti-trypsin revealed normal functions of human hepatocytes in the TRAIL-treated chimeric mice. These findings provide the first evidence that non-tagged soluble human TRAIL is not hepatotoxic to human livers in vivo.

*Conclusions*. Non-tagged human TRAIL kills glioma and melanoma cells, but not normal human astrocytes and hepatocytes in vitro. This TRAIL has a profound effect on tumor growth without causing hepatotoxicity in mice with chimeric human livers.

### 319P. Ubiquitin-Positive Inclusions in Ependymal Cells

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Ubiquitin-positive inclusions (UbIs) have not been well studied in ependymal cells yet. Since we detected such UbIs in the central canals of the medulla and spinal cord while investigating UbIs in neurodegenerative diseases, we studied the UbIs in the entire ependymal system of 42 patients with various neurological diseases and of 10 non-neurological controls. UbIs were located in the cytoplasm of the ependymal cells, and were round to oval in shape, measuring 4 to 11 μm in diameter. The UbIs were non-argyrophilic and undetectable by the hematoxylin and eosin staining, but mildly reactive to the periodic acid-Schiff stainings with and without digestion. The UbIs were variably immunoreactive for anti-epithelial membrane antigen (EMA) antibody, but did not react with various other antibodies. The co-existence of ubiquitin and EMA was confirmed by confocal laser microscopy. Throughout the ependymal system, UbIs were variably found in ependymal cells as well as in subependymal cells. There was no significant difference in the overall incidence of either ependymal or subependymal UbIs between the neurological patients and controls. However, ependymal UbIs in the central canal were more frequent in the neurological patients than in controls, although there was no disease specificity. This is the first comprehensive report to show common occurrence of UbIs in the ependymal cells of adult human brains.

### 320. 6-Hydroxydopamine Causes Apoptosis of Dopaminergic Neurons in Hemiparkinsonian Rats

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Main goal of this study was to investigate the apoptotic pathway involved in degeneration of dopaminergic neurons (DA) in Parkinson's disease. Programmed cell death was examined in striatum and substantia nigra (SN), after lesioning right side of striatum by the 6-hydroxydopamine (6-OHDA), at postoperative time points 6 hours, 24 hours and 7 days. Using TUNEL technique, we analyzed the induction of apoptotic nuclei in the striatum and SN or by RT-PCR method we followed the level of Bax mRNA expression.

At early postoperative time points (6 and 24 hours) we observed significant increase of Bax mRNA expression (40-45%) in the ipsilateral striatum of treated animals in comparation to the right striatum of control animals. However, the highest level of the Bax mRNA expression was reached 7 days after the surgery (94%) in the ipsilateral striatum of 6-OHDA treated animals.

In situ analysis revealed increased number of TUNEL positive neurons in 6-OHDA treated animals at all time points examined. The highest number of apoptotic neurons was detected 24 hours after the lesion, both in the striatum ($3.41 \pm 0.18$) and in the SN ($5.8 \pm 0.79$). Our results suggest that 6-OHDA induce early striatal changes that can trigger apoptotic pathway of DA neurons, during the first post-operative week.

## WORKSHOPS

### Development of Oligodendrocytes

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The oligodendrocyte lineage develops from restricted subsets of neuroepithelial precursor (stem) cells in the embryonic neural tube. In the spinal cord, for example, oligodendrocyte progenitor cells (OPCs) arise in a small ventral domain of the ventricular zone (VZ) called pMN, then migrate throughout the cord before differentiating into myelinating oligodendrocytes. pMN first generates motor neurons before switching to OPCs. This link between motor neurons and oligodendrocytes is puzzling, but might reflect some evolutionary connection between motor axon ensheathment and locomotion. In the developing forebrain, OPCs originate in the ventral VZ and apparently migrate dorsally to the cerebral cortex. There might also be a source of OPCs in the cortex.

Sonic hedgehog (SHH) signalling is required for induction of OPCs in the ventral VZ. FGF2 can also specify OPCs in culture but it is not known how, or whether, FGF2 and SHH signalling pathways are interrelated. SHH and FGF2 both induce expression of the transcription factors Olig1 and Olig2, which are crucial for OPC induction. Olig1/Olig2 double knockout mice completely lack oligodendrocytes (and motor neurons).

Large numbers of OPCs persist in the adult CNS. These cells can revert to a multipotent fate and can generate neurons and astrocytes as well as oligodendrocytes in vitro. It is not known whether they display this stem cell-like behaviour in vivo, during normal life or following CNS injury or disease.

I shall provide an overview and update of our work relating to the above.

### Pathology and Genetics of Oligodendroglioma

von Deimling A, MD

Novel findings in oligodendroglioma will be addressed in this update:

The threshold for separating oligodendroglioma and oligoastrocytoma from astrocytoma has always varied considerably between individual neuropathologists, now is further aggravated by the description of oligodendroglioma with neurocytic differentiation. However, this finding contributes to the discussion, whether oligodendrogliomas arise from precursor cells with glial determination or whether these tumours may be derived from a more pluripotent cell capable of neuronal differentiation.

The typical molecular fingerprint of oligodendroglial tumours is combined LOH1p and LOH19q. These deletions have been shown to be prognostically favourable indicators of better survival and response to chemotherapy in patients with anaplastic oligodendroglial tumours. The prognostic power of these molecular alterations was found to be even superior to histological and immunohistochemical findings. Less fallible to individual interpretation, genetic alterations may be a useful tool to assist in classification and therapeutic decisions.

Exclusive loss of paternal alleles on 19q in oligodendrogliomas has been reported, indicating parental imprinting to play a role in these tumours. If supported, these findings would alter the strategy for identifying the putative tumor suppressor gene. We, therefore, analyzed DNAs from thirteen oligodendroglial tumors with LOH 19q for parental origin of the lost alleles. Lost alleles on 19q were of maternal origin in 7 and of paternal origin in 6 cases, representing random distribution. While methylation as an important epigenetic mechanism for gene regulation always needs to be considered, classical analysis for inactivating mutations on chromosome 19q should not be discontinued in oligodendrogliomas.

### Murine Models of Oligodendrogliomas

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Gliomas constitute the most common and most lethal class of primary human brain tumor. Among the various traditional histopathologic categories of glioma, classical oligodendroglioma (OL) and anaplastic oligodendroglioma (AO) are distinguished at the morphologic level by highly stereotypical phenotypic characteristics and at the molecular genetic level by an association between therapeutic responsiveness and deletions involving chromosomes 1p and 19q. Until very recently, the only murine models for study of OL/AO were xenografts of human tumors. Although xenograft-based murine models still have a role to play, an increasing number of primary murine oligodendroglioma model systems are now available. These models provide more precise phenotypic replicas of human tumors in terms of critical biologic features such as diffuse parenchymal invasion, and also allow for the high degree of flexible and precise genetic manipulation of specific precursor cell populations that is required for many types of modern investigations, such as the molecular dissection and recreation of oncogene pathways involved in glioma initiation and progression. Current OL/AO models offer unparalleled opportunities not only for study of basic genetic underpinnings but also of preclinical experimental therapeutic intervention investigations. In regard to the latter studies, the full panoply of contemporary biomedical technology has been brought to bear on murine OL/AO models, including small animal magnetic resonance (MR) imaging and in vivo biomarker techniques for tumor detection and monitoring.

## PLATFORM PRESENTATIONS

### 321. Clinicopathologic Studies of Oligodendrogliomas Revealing Features of Neuronal Differentiation

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Recently a group of tumors has been described which morphogically mimic oligondendrogliomas (OL): neurocytomas (NC) and dysembryoplastic neuroepithelial tumors (DNT). Recent papers have revealed that some OLs, both immunohistochemically and ultrastructurally, may exhibit features of neuronal differentiation. In order to investigate this form of OL, we examined 64 differentiated and 6 anaplastic OLs. We found that a variable immunoreactivity for neuronal antigens was present in 40% of the cases. In addition, we studied a "small cell malignant brain tumor (SCMBT)" in which there were some small oligo-like areas and islands of cells positive to neuronal antigens intermingled with other areas with cells positive to GFAP. Upon ultrastructural analysis both areas showed oligo-like cells containing aggregates of intermediate-glial filaments; only few cells featured electrodense neurosecretory granules. Focally synaptic-like cytoplasmic projections were recognizable. Genetic analysis of areas of cells GFAP positive showed a 10q deletion. Our diagnosis was, "Anaplastic oligoastrocytoma with neurocytic differentiation."

Our findings in these tumors support an association between glial and neuronal ontogeny and imply that glial tumors might arise from a single glioneuronal progenitor. This cell could be the pluripotential embryonic stem cell (ESC) grown in culture in the nascent blastocystic stage. The ESC forms teratomas in vivo and can differentiate progenitors of neural stem cells (Noggin cells) and from them, mature neurons, astrocytes and oligodendrocytes, thus linking astrocytic tumors, oligodendrogliomas and neurocytic tumors.

### 322. Tumor Necrosis and Microvascular Proliferation are Associated with 9p Deletion and CDKN2A Alterations in 1p/19q-Deleted Oligodendrogliomas

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[4] Institute of Neurology, London, United Kingdom.

*Background*. Oligodendrogliomas and oligoastrocytomas have been associated with 1p/19q deletion, a alteration subsequently linked to chemosensitivity and oligodendroglioma's classic histology. Tumoral progression includes deletions of 9p, 10q and alterations of CDKN2A. However, these (epi)genetic changes have not been associated with specific histological features.

*Methods*. In a series of 45 gliomas including oligodendrogliomas, oligoastrocytomas and astrocytomas, we looked for 1p, 9p, 10, 17p13, 19q and 22 deletions by microsatellite analysis and for p14ARF, CDKN2A and CDKN2B (epi)genetic alterations by methylation specific PCR and sequencing.

*Results*. Deletion of 1p/19q was observed in 22 tumors, 21 presenting regions of oligodendroglioma's classic histology. An additional 9p deletion was found in 8, always in association with tumor necrosis and/or microvascular proliferation. In addition, (epi)genetic alterations of CDKN2A were observed in 71% of them.

*Conclusions*. *i)* Presence of regions of classic histology of oligodendroglioma in a tumor sample is predictive of 1p/19q-deletions, *ii)* necrosis and/or microvascular proliferation are signs of an additional 9p deletion, and *iii)* as CDKN2A (epi)genetic alterations were found in 71% of the 1p/19q/9p-deleted oligodendrogliomas, CDKN2A may have a role in oligodendroglioma associated microvascular proliferation.

### 323. Cyclins and CDKs in Classic and Anaplastic Oligodendrogliomas

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Cyclin D1 is not overexpressed in malignant gliomas. However, in astrocytic gliomas cyclin A, B1, D1 and E increase with malignancy. In oligodendrogliomas, the study of cyclins is complicated by the positive staining for cyclin D1 of normal oligodendrocytes and microglia cells. In a series of 23 operated oligodendrogliomas cyclin D1, A, E and B1, cyclin dependent kinases 2 and 4 and cdc2 were studied by the relevant antibodies and the LI was calculated for each of them in areas with the highest expression. The LI of Ki.67 MIB-1 was calculated as well. Oligodendrogliomas included 12 tumors grade II and 11 tumors grade III, according to WHO. Cyclin D1 and A showed a LI definitely higher in malignant than in classic oligodendrogliomas. Cyclin B1 and E were rarely positive, but in malignant cases only. Kinases were positive in most malignant tumors and in some grade II tumors. The increase of the LI for cyclins with anaplasia parallelled that of MIB-1 LI. Above an adequate cut-off, it could indicate malignancy also in individual cases, especially when associated with other parameters. Kinases could have a prognostic significance only in cases of positive staining. A differentiation of tumor oligodendrocytes from normal oligodendrocytes in peripheral areas may be supported by the negative staining of the latter for cyclin A and a positive staining for cyclin D1.

### 324. Neurocytoma and Dysembryoplastic Neuroepithelial Tumor are Genetically Different from Oligodendroglial Tumors

Fujisawa H; Marukawa K; Hasegawa M; Hayashi Y; Tachibana O; Yamashita J

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*Object*. Because of their histological similarities, it is occasionally difficult to differentiate neurocytoma and dysembryoplastic neuroepithelial tumor (DNT) from oligodendroglial tumors. Allelic loss on 1p and 19q and p53 gene mutation was analyzed in these tumor types.

*Methods*. A total of 24 cases were analyzed comprising 8 central neurocytomas, 3 DNTs, 7 oligodendrogliomas, 4 oligoastrocytomas and 2 extraventricular tumors with neurocytoma features (ETNF). Allelic loss was determined by microsatellite analysis. A p53 mutation was identified by PCR-SSCP analysis and direct sequencing. Immunohistochemistry of synaptophysin and electron microscopy were also conducted. Allelic loss on 1p and 19q was detected in 6 oligodendrogliomas (86%) and 3 oligoastrocytomas

(75%), but in none of the neurocytomas or DNTs. A p53 missense mutation was detected in only one oligoastrocytoma without allelic loss. Synaptophysin was expressed in all central neurocytomas and DNTs, 3 oligodendrogliomas (43%) and 3 oligoastrocytomas (75%). Of the ETNFs, one demonstrated synaptophysin expression and neural ultrastructures but lacked genetic alterations, while the other showed allelic loss on 1p and 19q but was negative immunohistochemically and ultrastructurally. The former was diagnosed as a potential intraparenchymal neurocytoma and the latter as an oligodendroglioma.

*Conclusions*. Despite histological similarities, central neurocytomas and DNTs are genetically distinct from oligodendroglial tumors. Examination for allelic loss on 1p and 19q and for p53 mutation can be useful for such distinction.

## POSTERS

### 325P. Correlation of Non-specific Alkaline Phosphatase Expression with LOH 1p Status in Oligodendrogliomas

Medvedev G; Horsman D; Moore W; McNeil K; Maguire J

*Introduction*. Loss of heterozygosity (LOH) of chromosome 1p in oligodendrogliomas has correlated with chemotherapeutic response, and been associated with higher histological grade and increased tumour recurrence in meningiomas. The non-specific alkaline phosphatase (NS-ALP) gene is located in the region 1p36-1p34: lack of expression of NS-ALP in meningiomas has correlated with increased propensity for tumour recurrence. Correlation between LOH 1p and absent NS-ALP expression has not been studied in gliomas. Our objective was to correlate NS-ALP immunohistochemical profiles of oligodendrogliomas with LOH 1p status.

*Methods*. LOH 1p status of 15 samples of 14 oligodendrogliomas was analysed using PCR and FISH. Corresponding sections of paraffin-embedded tissue were assessed by immunocytochemistry for NS-ALP employing a monoclonal antibody (US Biologicals, 1/100, protease) and studied in a double-blinded manner. Results: Seven oligodendrogliomas had LOH 1p, seven oligodendroglioma samples had ROH 1p. Three patterns of NS-ALP immunohistochemical staining within the tumours were observed: *i)* membranous, *ii)* cytoplasmic, and *iii)* discrete areas of neuropil (within tumour). All ROH 1p samples displayed one or more of these patterns of NS-ALP expression. 2/7 LOH 1p samples were positive and 5/7 LOH 1p samples were negative for NS-ALP expression.

*Conclusions*. These data indicate lack of NS-ALP expression tends to be associated with LOH 1p, although the correlation is not absolute. Therapy-related decisions concerning LOH 1p status should therefore be based on proven molecular genetic techniques rather than the possible loss of expression of products whose genes may be inconsistently incorporated in the region of the deletion.

### 327P. The OLIG2 Gene Encodes a Basic Helix-Loop-Helix Transcription Factor

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The OLIG2 gene encodes a basic helix-loop-helix transcription factor involved in the specification of the oligodendroglial lineage. Using a newly developed anti-Olig2 antibody, immunostaining done in normal brain, 6 oligodendrogliomas and 12 non-oligodendroglial or non-astrocytic brain tumors demonstrated that Olig2 protein was a specific nuclear marker of normal and tumoral oligodendrocytes. In 20 diffuse astrocytomas, all MIB positive tumoral cycling cells co-expressed Olig2 but not GFAP. These results strongly suggest that diffuse astrocytomas are infiltrative forms of oligodendrogliomas that induce a major reactive astrogliosis of the involved parenchyma. Olig2 immunodetection will implement a reproducible classification of gliomas, crucial for clinicians and scientists.

### 328P. Establishment of Anti-human Olig2 Antibody and Successful Application to Detect Human Oligodendrocytes and Oligodendroglial Tumors on Formalin-Fixed, Paraffin-Embedded Tissue Sections

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Olig2 is a recently identified transcription factor involved in the phenotype definition of cells in the oligodendroglial lineage. The expression of Olig2 transcript has been demonstrated in human oligodendroglial tumors, although the state of protein expression is unknown. We developed a polyclonal antibody to human Olig2 using a synthetic peptide, and analyzed it immunohistochemically. Western blotting showed a single band of predicted molecular weight. By immunohistochemistry using formalin-fixed, paraffin-embedded normal human brain tissue, the nuclei of oligodendrocytes of interfascicular, perivascular and perineuronal disposition were clearly labeled by the antibody. Similarly, the nuclei of oligodendroglial tumors were labeled.

There was no apparent correlation between the staining intensity and histological grade. Astrocytic components within the tumors were generally less or not stained. The expression of Olig2 transcript was confirmed by reverse transcription—polymerase chain reaction using RNA extracted from adjacent paraffin sections. Neither central neurocytoma nor schwannoma cases were stained by the Olig2 antiserum. Our antibody was demonstrated to be quite useful in recognizing normal and neoplastic oligodendrocytes on paraffin sections.

## PLATFORM PRESENTATIONS

### 329. Molecular Pathology of Meningeal Hemangiopericytomas I. p53 Pathway Alterations: HDM2 Overexpression and $p14^{ARF}$ Focal Loss of Expression

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p53 pathway expression ($p14^{ARF}$, p53, $p21^{WAF1}$, HDM2) and Ki67 proliferation index (PI) was studied in 11 meningeal hemangiopericytomas (MHPCs), 4 of them recurrent in 1, 2 and 4 occasions (18 specimens).

Ki67 index ranged from 2.15 to 10.96%; mean and 95% confidence interval (M ± 95% CI): 4.81 ± 1.76 for primary tumors: 3.49 ± 1.02 in non-recurrent cases and 7.13 ± 3.7 in recurrent ones. The PI in recurrent cases increased along progression.

All cases displayed simultaneous p53 and wild-type p53 transactived genes $p21^{WAF}$ and HDM2 protein expression. This argues against p53 mutation. HDM2 levels ranged between 0.9 and 22.21% (Mean ± 95% CI =6.43 ± 4.02): 4.60 ± 2.78 non-recurrent cases and 9.64 ± 10.04 recurrent. A tendency to HDM2 increase was show along recurrences. Thus HDM2 overexpression could contribute to MHPC's progression, consistent with p53 pathway alteration by a different mechanism than p53 point mutation.

$p14^{ARF}$ expression varied from 1.09 to 21.48 (Mean ± 95% CI = 6.23 ± 3.3) in all primary cases; 7.37 ± 4.98 for non-recurrent cases and 4.25 ± 2.28 for recurrent. Focal loss of expression was observed in 3 primary cases and in 2 successive recurrences, usually associated to anaplastic areas.

In conclusion, p53 pathway deregulation by HDM2 overexpression, sometimes combined with $p14^{ARF}$ focal loss, may be pathogenic for MHPCs.

### 330. Diagnostic Utility of CD10 in Discriminating Hemangioblastoma from Metastatic Renal Cell Carcinoma in the Central Nervous System

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*Introduction*. Hemangioblastoma (HB) is a rare benign highly vascular tumor of the central nervous system (CNS). The differential diagnosis between hemangioblastoma and metastatic clear cell type renal cell carcinoma (RCC) can be problematic, because there may be striking histologic similarities between them. CD10 is a 100 kd cell surface neutral metalloendopeptidase. Recent studies demonstrated that CD10 is a highly specific marker for RCC. To our knowledge, these findings have not been explored in HB. The aim of the present study was to evaluate the staining pattern of CD10 in HB and RCC with emphasis its possible usefulness in the differential diagnosis.

Materials and methods. Formalin fixed, paraffin embedded tissues of twenty HB, 5 metastatic RCC in the CNS, and 16 primary kidney RCC were retrieved from the pathology file of Chang Gung Memorial Hospital. Immunohistochemical study was performed using a monoclonal antibody to CD10. Intensity and percentage of membrane staining were evaluated.

*Results*. All twenty HB cases showed immunonegativity for CD10 without membrane staining in the stromal cells. All 5 metastatic RCC and 16 primary RCC showed positivity for CD 10 with moderate to strong membrane staining.

*Conclusions*. CD 10 is a useful marker in distinction between HB and metastatic RCC in the CNS. In addition to the use of epithelial membrane antigen, CD 10 immunostaining can be applied to the differential diagnosis between these neoplasms.

## WORKSHOPS

### Peripheral Distribution of $PrP^{Sc}$ in Human Prion Diseases

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*Background*. Human prion diseases are rare fatal neurodegenerative conditions which occur as acquired, familial or idiopathic disorders. A key event in their pathogenesis is the accumulation of an altered form of the prion protein, termed $PrP^{Sc}$ in the central nervous system. A novel acquired human prion disease, variant Creutzfeldt-Jakob disease, is thought to result from oral exposure to the bovine spongiform encephalopathy agent. This disease differs from other human prion diseases in its neurological, neuropathological and biochemical phenotype.

*Methods*. We have used immunohistochemistry and Western blot techniques to analyze the tissue distribution and biochemical properties of $PrP^{Sc}$ in peripheral tissues in a unique series of variant Creutzfeldt-Jakob disease cases. We have compared this with the distribution and biochemical forms found in all of the major subtypes of sporadic Creutzfeldt-Jakob disease and in iatrogenic Creutzfeldt-Jakob disease associated with growth hormone therapy.

*Results*. Involvement of the lymphoreticular system is a defining feature of variant Creutzfeldt-Jakob disease, but that the biochemical isoform of $PrP^{Sc}$ found is influenced by the cell type in which it accumulates.

*Conclusions*. Variant CJD differs from other human prion diseases in terms of lymphoid tissue involvement. This may reflect the peripheral pathogenesis of this acquired disease, and raises concerns about potential iatrogenic transmission by surgical procedures on these tissues.

### Peripheral Prion Distribution in Natural Scrapie, BSE and Chronic Wasting Disease

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Several studies have shown that, in BSE, scrapie and chronic wasting disease, prion infection can be detected in various peripheral tissues before neuroinvasion occurs. The main tissues in which the presence of infection has been detected include lymph nodes, spleen, gut-associated lymphoid tissue (GALT, including Peyer's patches), tonsil and nictitating membrane, although there are some qualitative and quantitative differences between cattle, sheep and deer. Within these tissues, $PrP^{Sc}$ is found chiefly associated with Follicular Dendritic Cells (FDCs) within the lymphoid follicles, although some PrPSc has also been identified in association with certain macrophage populations. The FDCs probably act as a site of amplification of infectivity within the peripheral tissues. This distribution of $PrP^{Sc}$ and infectivity can be reproduced following experimental oral challenge of these animal species, suggesting that a systemic spread of infectivity occurs soon after oral infection. However, time course studies following such challenge in natural hosts or in rodent models have shown that neuroinvasion from these peripheral tissues occurs via their sympathetic and/or parasympathetic innervation rather than by a haematogenous route. Indeed, the role (if any) of "prionaemia" in animal-to-animal transmission in naturally-occurring disease remains unclear. Although $PrP^{Sc}$ (and infectivity) in peripheral tissues is found mostly in lymphoid organs, other tissues—notably the intestinal mucosa—may also harbour infectivity and it is thought that under circumstances of high infective doses, neuroinvasion can occur directly via the innervation of these tissues without prior amplification in lymphoid follicles. Later in disease progression, low levels of infectivity may be detected in other organs, possibly as a result of a spread of infection from the CNS down peripheral nerves.

### Peripheral Routing of Infection in Orally Acquired Transmissible Spongiform Encephalopathies

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*Background*. Hamsters fed with scrapie provide an animal model that during the past few years has revealed key pathogenetic features of the spread of infection through the body in orally acquired transmissible spongiform encephalopathies (TSEs).

*Methods*. Using $PrP^{Sc}$ as a biochemical marker for infectious TSE agent, and immunohistochemistry, Western- and paraffin-embedded tissue (PET) blotting for its detection, it was possible to map the temporal-spatial spread of infection from the alimentary tract to the central nervous system (McBride et al, *J Virol*, 75:9320-9327).

*Results*. This revealed early infection of the gut-associated lymphoid tissue and the enteric nervous system following ingestion of infectivity. Subsequently, the agent invades initial target sites in the brain and spinal cord in a defined temporal sequence via synaptically linked autonomic ganglia and efferent fibres of the vagus and splanchnic nerves, respectively. Furthermore, at the terminal stage of the disease, skeletal muscles of hamsters orally infected with scrapie show substantial accumulations of $PrP^{Sc}$ (Thomzig et al., 2003, EMBO reports, 4, 530-533).

*Implications*. As similar pathogenetic mechanisms may also operate in naturally occurring scrapie of sheep and goats, field cases of bovine spongiform encephalopathy (BSE) in cattle, and orally transmitted or naturally occurring chronic wasting disease (CWD) of elk and deer, the findings in our animal model highlight the necessity to investigate thoroughly the TSE risk in the autonomic nervous system and muscles of these species.

### Abnormal PrP Isoforms in Urine

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Since the appearance of BSE and subsequently vCJD, the need for an in vivo diagnostic test for prion diseases has become acute. vCJD is a fatal neurodegenerative disease believed to be caused by the consumption of BSE contaminated meat, and the incubation time between infection to clinical symptoms may be as long as decades. The incubating individuals, (which at this point can be any of us), are going to be present for many years, donating blood and interacting with the community. For diverse reasons, it is difficult to speculate on the number of future vCJD patients.

Protease resistant PrP ($PrP^{Sc}$) is the only universally accepted marker for prion diseases, and can be easily detected in the brains of animals and humans affected with these diseases. We have

found that a protease resistant form of PrP ($UPrP^{Sc}$) can also be found in the urine of scrapie infected hamsters, BSE infected cattle and humans suffering from Creutzfeldt-Jakob disease. In addition to its presence in the urine of terminally ill prion infected hamsters, UPrPSc was also detected in hamsters as early as 35 days after intra cerebral inoculation with prions, long before the appearance of clinical signs of the disease. Similar results were obtained for hamsters after intraperitoneal infection with prions.

The properties of the urine PrP isoforms differ from those of the brain PrP isoform. $UPrP^{Sc}$ is less protease resistant, less aggregated and binds lightly to a resin loaded copper, as opposed to $PrP^{Sc}$ which is not retained by the same resin at such conditions. In addition, inoculation of $UPrP^{Sc}$ to hamsters suggest scrapie urine comprises low levels of prion infectivity.

### $PrP^{res}$ Amplification: Perspectives of a Diagnostic Test In Vivo

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$PrP^{Sc}$, the major component of prion infectious agent, replicates in vivo at expenses of the cellular prion protein ($PrP^{C}$) during the long asymptomatic phase that follows infection. We have recently developed a technology named "Protein Misfolding Cyclic Amplification (PMCA)," that mimics in the test tube the process of prion replication. Undetectable amounts of $PrP^{Sc}$ are incubated with a large excess of $PrP^{C}$ to allow the conversion of the normal protein. The process is amplified by cycles of sonication in order to multiply exponentially the number of converting units. As a result, the amount of $PrP^{Sc}$ is dramatically increased, simplifying its detection. PMCA
is very specific to amplify prions, because in the absence of the abnormal protein, $PrP^{C}$ is not converted.

These findings mark the first time in which the folding and biochemical properties of a protein have been cyclically amplified in a manner conceptually analogous to the amplification of DNA by PCR. PMCA has enormous potential in allowing current diagnostic tools to detect BSE and vCJD at a much earlier stage, even during the pre-symptomatic period. PMCA opens the door to detecting the disease in living individuals using blood or peripheral tissue samples. In addition, PMCA represents a good tool to help understanding the underlying biology of prions, to identify other factors that may be responsible for prion protein conversion, and to discover novel drug targets for prion diseases.

## PLATFORM PRESENTATIONS

### 332. Gerstmann-Sträussler-Scheinker Disease Associated with the PRNP P102L-129V Haplotype

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*Introduction*. There are 2 known cases of Gerstmann-Sträussler-Scheinker disease associated with the PRNP P102L-129V haplotype that are homozygous for valine at residue 129 of the prion protein (PrP). We previously reported one of these individuals who had seizures at age 33 and developed numbness of the lower extremities and difficulty with his gait at age 36. He became dysarthric and had difficulties swallowing. He died 12 years after clinical onset. According to the clinical history obtained from family members, the patient did not have dementia.

*Methods*. Neuropathologic studies were carried out using histology and immunohistochemistry.

*Results*. The main pathologic alterations are PrP-immunolabeled deposits that appear as synaptic-like structures or plaques similar to those associated with the PRNP P102L-129M haplotype. The plaques are fluorescent in thioflavin S preparations and therefore, are believed to be deposits of PrP-amyloid. PrP deposits are abundant in the cerebral and cerebellar cortices. Amyloid is abundant in the lower layers of the neocortex and in the cerebellar molecular layer. PrP deposits are also seen in the colliculi, substantia nigra, inferior olivary nuclei, and substantia gelatinosa. PrP immunopositivity is associated with the walls of some parenchymal vessels. There is a severe loss of fibers in the corticospinal, spinothalamic and gracile tracts. Spongiform changes are not present.

*Conclusions*. The present study extends our understanding of the neuropathology of Gerstmann-Sträussler-Scheinker disease. (P30 AG10133).

### 333. Neuropathological Characteristics of Brainstem Lesions in Sporadic Creutzfeldt-Jakob disease

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There have been a few reports of brainstem lesions in patients with Creutzfeldt-Jakob disease (CJD), but CJD associated brainstem abnormalities are considered uncommon. We examined whether the brainstem is affected by pathological processes, especially prion protein (PrP) deposition, in patients with CJD. To establish the presence of brainstem lesions in cases of CJD, we investigated PrP deposition and the brainstem atrophy in 30 autopsied patients with sporadic CJD. Paraffin-embeded brainstem sections were immunostained with monoclonal antibody against PrP by the EnVision method. Brainstem atrophy, especially in the pontine base, was relatively prominent in individuals with prolonged disease. The motor nuclei of the brainstem were relatively well preserved. PrP deposition was present, mainly in the substantia nigra, quadrigeminal bodies, pontine nucleus, and inferior olivary nucleus. It was less dense in the red nucleus and tegmentum of the brainstem and it occurred least in the pyramidal tract of the medulla oblongata, longitudinal fasciculus of the pons and cerebral peduncles of the midbrain. PrP deposition was moderate in the in substantia grisea centralis surroundings the oculomotor nerve nucleus and locus ceruleus. The density of PrP deposition in the brainstem seemed to correlate with disease duration.

We conclude that widespread PrP deposition in the brainstem is an important pathological feature of CJD, and the brainstem may be relatively resistant to the CJD disease process.

### 334. Sporadic Creutzfeldt-Jakob Disease: Deposition of Protease Resistant Prion Protein in the Retina

Mangieri M; Giaccone G; Capobianco R; Limido L; Di Fede G; Suardi S; Rossi G; Fociani P; Carsana L; Tagliavini F; Bugiani O

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*Background*. Creutzfeldt-Jakob disease (CJD) is characterised by the presence of the proteinase K-resistant prion-protein ($PrP^{res}$) in the brain.

*Methods*. We analyzed for $PrP^{res}$ the retina from 27 sporadic CJD patients (PRNP codon 129 polymorphism: MM n = 22, VV n = 3, MV n = 2) and 15 patients with other neurological diseases. The diagnosis of CJD was definite on the basis of neuropathological findings, presence of cerebral $PrP^{res}$ deposition by immunohistochemistry and Western blot analysis, absence of PRNP mutations. The ocular tissue was collected at autopsy and fixed in Carnoy solution. Before immunohistochemistry with 3F4 antibody, the sections were pretreated with proteinase K and guanidine thiocyanate.

*Results*. $PrP^{res}$ immunoreactivity was detected in the retina of all 27 sporadic CJD patients, but not in control patients. In CJD, $PrP^{res}$ was localized in the outer and inner plexiform layers of the retina, with a coarser granular pattern in the former, without differences between patients with diverse polymorphism at codon 129 of PRNP. Western blot analysis confirmed the presence of PrPres, whose relative amount, protease-resistance and molecular weight were identical to those of the brain.

*Conclusions*. Our data indicate that $PrP^{res}$ deposition in the retina is a consistent event in sporadic CJD.

### 335. Vintage 2003: Diagnosis of Creutzfeldt-Jakob Disease by Olfactory Epithelium Biopsy

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In sporadic Creutzfeldt-Jakob disease (sCJD) there are no assessed peripheral markers for use in the diagnosis of the disease in living patients. Definitive diagnosis requires pathological examination of the brain and demonstration of the abnormal prion protein isoform ($PrP^{Sc}$) by immunohistochemistry or Western blot. Recently, we reported the presence of $PrP^{Sc}$ in the olfactory system, including the olfactory mucosa, in post-mortem specimens from sCJD victims. Strikingly, $PrP^{Sc}$ deposition was found also in subjects with short-duration disease, thus suggesting that olfactory biopsies might provide an early diagnosis in suspected sCJD.

Here we show preliminary results obtained in 2 patients with clinical diagnosis of probable/possible sCJD. Multiple olfactory mucosa specimens, obtained after informed consent, were processed for immunohistochemical studies, and, tentatively, for immunobiochemical analysis, which however, in our hands, requires large amounts of olfactory tissue.

By immunocytochemistry we observed $PrP^{Sc}$ deposition at the level of olfactory cilia and basal cells in both cases, while, as expected, the limited quantity of tissue available for Western blot, precluded a biochemical identification of $PrP^{Sc}$; importantly, one case was confirmed six months later at autopsy. Current efforts are devoted to increase the sensitivity of the methods for immunobiochemical $PrP^{Sc}$ detection.

### 336. Prion Protein Accumulation Involving the Peripheral Nervous System in Some Sporadic Cases of Creutzfeldt-Jakob Disease

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*Background*. There is increasing evidence that the spinal cord and peripheral nervous system (PNS) are involved in the pathogenesis of some forms of prion diseases. We searched for prion protein (PrP) accumulation involving the PNS in 7 cases of sporadic Creutzfeldt-Jakob disease.

*Methods*. Autopsy specimens from cerebral cortex, cerebellum, spinal cord, sciatic and peroneal nerves were paraffin embedded, and PrP immunostaining was performed with 3F4 and 12F10 monoclonal antibodies after proteinase K (PK) digestion. Frozen specimens from the same areas were homogenized and PK treated for Western-blot analysis.

*Results*. PrP immunostaining detected PrP accumulation in cerebral cortex from 7 of 7 cases, in cerebellar cortex from 6 of 7, and in the spinal cord from 7 of 7. PrP accumulation was also found in the sciatic nerve from 2 of 7 cases and in the peroneal nerve from 3 of 7. Linear deposits were seen along nerve fibers; both myelinated and unmyelinated fibers appeared to be involved. Up to now, Western-blot analysis is completed in 4 cases. This evidenced PK resistant PrP accumulation in the cerebral cortex, the cerebellar cortex and the spinal cord from the 4 cases. PK resistant PrP was also found in the peroneal nerve from one case.

*Conclusions*. Our results confirmed involvement of the PNS in some sporadic cases of Creutzfeldt-Jakob disease.

### 337. Rise of CJD-Incidence in Switzerland: Phenotypic Spectrum

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Transmissible spongiform encephalopathies (TSEs) such as sporadic, familial and acquired Creutzfeldt-Jakob disease have been known to affect humans for centuries. An abnormal form of the prion protein ($PrP^{Sc}$) is thought to represent the principal constituent of the infectious agent. In Switzerland CJD is statutory notifiable since December 1987. Between 1996 and 2000, the incidence of CJD fluctuated between 1 to 1.4 cases per million inhabitants per year. A rise in incidence was reported in 2001 which persists in 2002, the incidence rate being around 2.7 (1). Genetic analysis revealed one disease-associated mutation in PRNP and the distribution of allelotypes on codon 129 is comparable to reported large sCJD collectives. None of the cases fulfill the diagnostic criteria for vCJD when assessed by internationally accepted criteria such as, glycotype profiling, histopathology, and clinical presentation.

Swiss CJD cases of the years 1996 to 2002 were assessed by biochemical methods and by comparing Swiss CJD cases to CJD cases from European and Non-European countries. Distribution of PrPSc in peripheral organs was assessed by a highly sensitive PrPSc detection method whereby $PrP^{Sc}$ is concentrated by sodium phosphotungstic acid precipitation and detected by western blotting (2).

1. Glatzel M et al (2002) Incidence of Creutzfeldt-Jakob disease in Switzerland. *Lancet* 360:139-141.
2. Maissen M et al (2001) Plasminogen binds to disease-associated prion protein of multiple species. *Lancet* 357:2026-2028.

## POSTERS

### 338P. The Epsilon Isoform of 14.3.3 is Present in Amyloid Deposits of Gerstmann-Sträussler-Scheinker Disease

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*Background*. The 14.3.3 proteins are highly conserved, ubiquitous molecules that are involved in numerous biological activities such as transduction pathways, cell cycle and apoptosis. Seven 14.3.3 isoforms have been identified. 14.3.3 proteins increase in the cerebrospinal fluid of patients with Creutzfeldt-Jakob Disease (CJD), where an isoform-specific pattern has been observed and its diagnostic value has been emphasized. Recently, a faint immunoreactivity for the zeta isoform of 14.3.3 has been found in PrP-amyloid deposits of patients with sporadic CJD.

*Methods*. An immunohistochemical study and immunoblot analysis was carried out on brain tissue samples from patients with sporadic CJD (n = 7), familial CJD (E200K = 1, V210I = 1), Gerstmann-Sträussler-Scheinker disease (GSS F198S = 3; GSS A117V = 1), Alzheimer Disease (AD) and other neurodegenerative disorders (n = 10), using specific antibodies against the seven 14.3.3 isoforms.

*Results*. The antiserum to the epsilon isoform of 14.3.3 strongly labelled PrP amyloid plaques in GSS, but not amyloid deposits in CJD nor senile plaques in AD. Conversely, no differences between GSS, CJD, AD and other neurodegenerative disorders were observed with antibodies against the other 14.3.3 isoforms, either by immunohistochemistry or Western blot analysis. In particular, no immunostaining of PrP amyloid was detected in CJD and GSS using the antiserum against the zeta isoform.

*Conclusions*. Our results indicate that the epsilon isoform of 14.3.3 is a component of amyloid deposits of GSS.

### 339P. Olfactory Involvement in Creutzfeldt-Jakob Disease

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We recently reported that the olfactory pathway is involved in the pathology of the classical and ataxic phenotypes of sporadic Creutzfeldt-Jakob Disease (sCJD). Here we performed a large-scale study in 44 sCJD subjects with different disease phenotypes and in 2 cases with familial CJD (fCJD). Patients were grouped according to the molecular type of the pathological prion protein ($PrP^{Sc}$) and the polymorphic codon 129 of the prion protein gene. In addition to conventional neuropathological examination, the pattern of PrP deposition and the regional $PrP^{Sc}$ distribution were determined in investigated brains.

In olfactory bulbs and tracts a granular type of PrP deposition was observed in all cases regardless of the $PrP^{Sc}$ type and 129 codon genotype, with a few cases showing intraneuronal deposition in the olfactory anterior nucleus. Conversely, olfactory cortexes showed a variable pattern of PrP positivity, ranging from synaptic-type to plaque-type deposition.

By quantitative Western blot, subjects valine homozygous or heterozygous at codon 129 with a 19-kDa protease-resistant $PrP^{Sc}$ type had the largest amounts of pathological prion protein in olfactory areas. $PrP^{sc}$ was however invariably detected in all investigated brains, including cases with short-disease duration and subjects with absent $PrP^{sc}$ in neocortical areas.

This study confirms that, similarly to Alzheimer's disease and Parkinson's disease, olfactory structures are involved in human prion diseases.

### 340P. Prion Protein Deposition in Peripheral Nervous System of Human Prion Diseases

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*Objectives*. To investigate abnormal prion protein (PrP) deposition in peripheral nervous system (PNS) of human prion diseases.

*Methods*. We examined 3 patients with sporadic Creutzfeldt-Jakob disease (sCJD), 2 with dural graft-associated CJD (dCJD), one with Gerstmann-Sträussler-Sheinker disease (GSS) with a PrP P102L mutation (GSS102), and 2 with a P105L mutation (GSS105) by immunohistochemical studies and western blot analyses. An atypical case of sCJD with PrP plaques clinically presented with peripheral neuropathy.

*Results*. In immunohistochemical studies with an anti-PrP monoclonal antibody (3F4), granular PrP deposited in some neurons of dorsal root ganglia (DRG) and a few fibers of peripheral nerves and spinal nerve roots in one sCJD and two dCJD patients, but not in GSS102 or GSS105 patients. The atypical case of sCJD with peripheral neuropathy showed demyelination in 12% of teased fibers, but no obvious PrP deposition in the nerves. Western blot analysis of the PNS from the CJD patients proved a small amount of PrPSc in the DRG.

*Conclusions*. Our results indicate that abnormal PrP deposition is not uncommonly found in the DRG and nerves of sCJD and dCJD, and that the PrP deposits in the PNS would not correlate with clinical presentation of peripheral neuropathy in CJD.

### 341P. Disease-Associated Prion Protein in Skeletal Muscle in Sporadic IBM

Kovacs GG[1,2]; Chimelli L[3]; Araújo AQC[4]; Ströbel T[1]; Glatzel M[5]; Aguzzi A[5]; Budka H[1]

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*Background*. Cellular prion protein ($PrP^{C}$) is essential in the formation of the pathological conformer, $PrP^{Sc}$, a hallmark of prion diseases affecting primarily the central nervous system. Recently, in experimental animals $PrP^{Sc}$ was demonstrated in muscle tissue. High expression of PrPC in muscle tissue from patients with sporadic inclusion-body myositis (s-IBM) is known as well.

*Methods*. Case report, PrP gene analysis, immunohistochemistry, paraffin embedded tissue blot (PET-blot) and Western blot to demonstrate $PrP^{Sc}$.

*Results*. We demonstrate $PrP^{Sc}$ in human deltoid muscle in a 64-year-old male patient with sporadic Creutzfeldt-Jakob disease (CJD) and s-IBM. The polymorphic codon 129 is methionine/valine heterozygous. We observe granular PrP immunoreactivity within muscle cells, supported also by PET blot, additionally to diffuse/synaptic and kuru-plaque PrP deposits in the brain. Western blot reveals $PrP^{Sc}$ in the brain and muscle. The relative content of PrP in muscle is 30% of that found in brain.

*Conclusion*. Co-occurrence of s-IBM and CJD might be associated with an increased chance for extraneural $PrP^{C}$-$PrP^{Sc}$ conversion as a consequence of a yet uncharacterized pathogenic event.

### 342P. Follicular Dendritic Cells as a Bioassay System for Human Prion Infections

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*Background*. Infectious prion diseases initiate infection within lymphoid organs where prion infectivity accumulates during the early stages of peripheral infection. In a mouse-adapted prion infection, an abnormal isoform ($PrP^{Sc}$) of prion protein (PrP) accumulates in follicular dendritic cells within lymphoid organs. Human prions, however, did not cause an accumulation of $PrP^{Sc}$ in the wild-type mice.

*Results and methods*. We produced knock-in mice with the homologous recombinatation technique and also transgenic mice with mouse PrP gene promoter. Here, we report that knock-in mouse expressing humanized chimeric PrP demonstrated $PrP^{Sc}$ accumulations in follicular dendritic cells following human prion infections, including variant Creutzfeldt-Jakob disease. The accumulated $PrP^{Sc}$ consisted of recombinant PrP, but not of the inoculated human PrP. These $PrP^{Sc}$ accumulations were detectable in the spleens of all mice examined 30 days post-inoculation. Infectivity of the spleen was also evident. However, the transgenic mouse epressing the same prion protein did not show the abnormal prion protein accumulations in the spleen, due to the attenuated PrP

expression in the follicular dendritic cells. This assay is valid for human prions with MM1, MV1, or MM2B, but not for MM2A, MV2, VV2.

*Conclusion*. This knock-in mouse model provides a new bioassay system against human infectious prion diseases.

### 343P. Diagnostic Usefulness of Urine Protein Analysis in Prion Diseases

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*Background*. Prion diseases are lethal transmissible neurodegenerative illnesses that affect humans and many other animals. Since the diagnostic criteria require the pathological confirmation of accumulation of abnormal isoform of prion protein in the central nervous system, early diagnosis of prion diseases in humans remains impossible. In 2001, Shaked et al reported the presence of protease-resistant prion protein in urine (UPrPSc) of animals and humans affected with prion diseases.

*Methods*. We collected urine from probable cases of prion diseases (n = 38), patients affected with Alzheimer's diseases (n = 20), non-demented disorders (n = 19) and also from healthy individuals (n = 21). Purified urine protein were digested with proteinase K and subjected to Western blot analysis.

*Results*. While 29 out of 38 patients with prion disease were positive for protease-resistant signal around 37 kDa on Western blot analysis, only 2 cases of non-demented patients were positive and none of patients with Alzheimer's disease or healthy individuals were positive for this test. However, these protease-resistant signals were also detected only with anti-mouse IgG antibody suggesting they were not specific for prion protein. Therefore, we conclude that detection of protease-resistant protein in urine is useful clinical test to support the diagnosis of prion diseases (76.3% in sensitivity and 96.0% in specificity), although this test does not provide the direct evidence of accumulation of abnormal isoform of prion protein.

### 344P. Prion Associated Increases in Src-Kinase

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*Background*. Prion diseases are characterized by spongiform degeneration, neuronal loss, and astrogliosis. Prions are aggregates of abnormal conformers of a normally expressed cell surface glycoprotein. The normal protein is designated $PrP^{c}$ (C for "cellular") and the disease-associated conformer as $PrP^{Sc}$ (Sc for "scrapie"). Conversion of $PrP^{c}$ to $PrP^{Sc}$ is fundamental for prion disease pathogenesis. How this molecular event results in the characteristic neuropathologic changes is unknown. Both $PrP^{c}$ and $PrP^{Sc}$ are concentrated in cholesterol and sphingolipid enriched membrane microdomains known as "rafts." Rafts sequester certain classes of proteins while excluding others. Rafts function in signal transduction, signal processing, and endocytosis. Levels of $PrP^{Sc}$ increase during the course of prion disease.

*Hypothesis*. Accumulation $PrP^{Sc}$ will correspond with the accumulation of other raft-associated proteins.

*Method*. Levels of the raft-associated src-kinase were examined by western blotting using cell or brain homogenates from N2a and ScN2a cells or 2 mouse models of prion disease (transgenic mice [Tg2866(MoPrP P101L)/PrPo/o] and Rocky Mountain Laboratory inoculated mice) respectively.

*Results*. Src-kinase was increased in all 3 model systems. Time-course studies in Tg2866(MoPrP P101L)/PrPo/o mice revealed a progressive increase in src-kinase which appeared shortly after the appearance of $PrP^{Sc}$, but before the onset of symptoms. Similar increases in src-kinase were not seen in end-stage mice over-expressing mutant APP.

*Conclusions*. These findings suggest increases in src-kinase are *i)* related to the presence of PrPSc, and *ii)* not a non-specific reactive epiphenomenon.

### 345P. Expression of Glutamate Transporter EAAT-1 by Activated Macrophages/Microglia (AMM) in Creutzfeldt-Jakob Disease (CJD) and Fatal Familial Insomnia (FFI)

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*Objectives*. The mechanisms of neuronal apoptosis in prion diseases is unclear. Experimental studies suggest that it may, at least partly, results from glutamate-mediated excitoxicity (Brown, *Microsc Res Tech* 54:71-80). Our group recently showed that AMM express glutamate transporters EAAT in HIV infection, suggesting that they may play a neuroprotective role by clearing extracellular glutamate (Decouvelaere, *J Neuropathol Exp Neurol* 62:5). We wished to test this hypothesis in prion diseases.

*Methods*. Samples from cerebral cortex, striatum, thalamus and cerebellum from 10 CJD patients (8 sporadic, 1 familial and 1 iatrogenic), 4 FFI patients (3 homozygous Met/Met at codon 129 of the PRNP, 1 heterozygous Met/Val), and 3 controls were immunostained for EAAT-1, GFAP, HLA-DR, CD68, caspase 3, and PrP.

*Results*. EAAT-1 immunopositivity was found in affected areas in prion diseases, and not in controls. Only AMM, interstitial, perivascular; perineuronal (sometimes around apoptotic neurons), or close to reactive astrocytes, expressed EAAT-1; astrocytes did not. Intensity of EAAT-1 expression did not correlate with that of neuronal apoptosis, spongiosis, astrocytosis, microgliosis, or PrP deposition, but with disease duration. Only occasional EAAT-1 expressing cells were found in patients with short survival (<3 months), whereas diffuse EAAT-1 expression by AMM was observed in cases with long survival (24-33 months) who most often were heterozygous Met/Val at codon 129 of the PRNP.

*Conclusion*. Our findings suggest that AMM, may play a neuroprotective role in prion diseases, particularly in long-lasting disorders. Whether the setting of a protective face of microglial activation is the cause or the consequence of longer survival needs to be clarified.

### 346P. Susceptibility to Various Human Prions of Knock-In Mice Expressing Chimeric PrP

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[3] CJD Surveillance Unit, Western General Hospital, Edinburgh, United Kingdom.

*Background*. Human prion diseases show clinicopathological diversity, reflecting the heterogeneity of the conformations of

pathogenic isoform of prion protein ($PrP^{Sc}$). They are classified based on etiology, polymorphism, methionine or valine, at codon 129 (129M and 129V, respectively), and $PrP^{Sc}$ types.

*Methods*. We produced a line of knock-in mouse expressing human-mouse chimeric prion protein designed to have mouse sequence only in the C-terminal part from 214 to 231 after post-translational modification (KiChM mouse). In order to assess the susceptibility profile of this mouse, we intracerebrally inoculated brain homogenates from 15 cases of human prion diseases with different etiologies, genotypes and $PrP^{Sc}$ types.

*Results*. Transmissibility to KiChM mice varied considerably between prions: prions with 129M and $PrP^{Sc}$ type 1 transmitted quite efficiently in ~150 days; those with 129M and $PrP^{Sc}$ type 2, either 2A or 2B, transmitted but with long incubation periods over 500 days; those with 129V and $PrP^{Sc}$ type 2 did not transmit.

*Conclusion*. KiChM mice had shorter incubation periods for some prions than the already reported transgenic mice, while less susceptible to other prions, presumably reflecting the varied compatibility of the chimeric prion protein for different $PrP^{Sc}$ conformations.

## WORKSHOPS

### Dopamine and Working Memory

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Working memory is a construct that describes the ability to transiently store and manipulate information on line to be used for cognition or for behavioral guidance. Numerous functional neuroimaging studies have shown that cortical areas like dorsolateral prefrontal cortex (DLPFC) and parietal cortex are centrally involved in working memory tasks. Working memory deficits are among the cardinal features of schizophrenia, and studies have shown a correlation between loss of neuronal functionality in DLPFC and reduction of working memory performance.

Dopamine transmission and signaling play a key role in the neurophysiology of DLPFC (probably determining the signal to noise ratio of glutamatergic neurons). In fact, previous studies in patients with schizophrenia have shown a correlation between reduction of cortical levels of dopamine in DLPFC and reduction of performance to working memory tasks. Moreover, another study has shown a relationship between genetically determined dopamine catabolism (the gene for catechol-o-methyl-transferase—COMT), performance to working memory tasks and cortical activation in DLPFC.

These data suggest a key role of dopamine in working memory that is relevant also from a clinical perspective for the treatment of schizophrenia. Second generation antipsychotic drugs seem to increase cortical levels of dopamine and this in turn might improve cortical functionality and working memory performance.

### Neuroimaging in Schizophrenia: Clinical and Research Applications

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Over the past 30 years the application of in vivo brain imaging techniques to the study of schizophrenia allowed the demonstration of various abnormalities of brain structure and function in the course of the disorder.

On the one hand, structural brain imaging techniques—such as computed tomography and magnetic resonance imaging—have consistently demonstrated the presence of ventricular enlargement, volume reduction of limbic and temporal cortical structures and loss of the normal cerebral asymmetries in the brain of schizophrenic patients.

On the other hand, functional neuroimaging techniques—such as single photon emission tomography (SPECT) and positron emission tomography (PET)—have shown metabolic deficits in frontal areas during rest conditions in schizophrenic patients when compared to healthy subjects and patterns of reduced or anomalous cortical activation during cognitive tests. Receptor binding studies in schizophrenia have been able to detect multiple anomalies of receptor systems, especially the dopaminergic system, and a different modulation of cerebral metabolism of atypical antipsychotic drugs as compared to conventional neuroleptics. Moreover, SPECT and PET techniques allowed the possibility to explain both therapeutic and side effects of antipsychotic drugs in relation to characteristics of receptor occupancy.

Most of functional magnetic resonance imaging studies in schizophrenia have shown patterns of different cortical activation between patients with schizophrenia and control subjects. These features have been replicated in several studies regardless the complexity of cognitive task and brain regions studied.

### Genetic Basis of Schizophrenia

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Schizophrenia is a severe mental disorder with a lifetime risk in the population of 1%. Like many common diseases, schizophrenia is multifactorial in origin, with both genetic and environmental factors playing an important role in the symptomatology. Family, twin and adoption studies have shown that schizophrenia has a high heritability suggesting that additive and interactive genes, each with small effects, mediate the genetic susceptibility for schizophrenia. The search for susceptibility loci using classical linkage studies suggested the involvement of several genomic regions but, at moment, not a single gene causing or predisposing to schizophrenia has been identified. The identification of genes having an influence on schizophrenia requires thus new strategies including specific statistical tools and the dissection of the heterogeneous syndrome using endophenotypes and/or relevant clinical feature.

In addition, a detailed analysis of the genes, using global expression studies by microarrays and new approaches in proteomics, can indicate good candidates for genetic association studies and, at the same time, providing molecular targets for the development of new and more effective therapies.

### Biological Bases and Molecular Pharmacology in Schizophrenia

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Schizophrenia is a complex and heterogeneous disease that, at the molecular level, can be characterized by anatomical defined alterations of several neurotransmitters including dopamine, serotonin and glutamate. However it is well accepted that events that interfere with the normal program of brain development may be relevant for the etiology of schizophrenia. Such events might ultimately determine a long-term impairment on brain function, which is characterized by enhanced vulnerability under challenging situations. In order to investigate molecular determinants for these events, we examined the modulation of the neurotrophin BDNF in response to developmental manipulations that produce long-term dysfunction resembling schizophrenia symptomatology. Under different experimental paradigms (ibotenic acid lesion, maternal deprivation), we found that the basal expression of BDNF was altered in selected brain regions including hippocampus and prefrontal cortex.

It is known that currently available antipsychotics mainly act as antagonists of different neurotransmitter receptors. However the latency for clinical amelioration suggests that different mechanisms contribute to their therapeutic activity. Indeed these drugs may

produce some of their effects through the regulation of neuroplastic markers, including neurotrophic factors, which could normalize the alterations produced by adverse events during brain development. We propose that neurodevelopmental animal model of schizophrenia may help to identify molecular players contributing to schizophrenia dysfunction, which may become the target for the development of novel and more effective drugs for the treatment of this psychiatric disorder.

### A Neurocognitive Approach to Schizophrenia

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*Background*. Cognitive dysfunction in schizophrenia is present at the onset of illness, in virtually all patients and in non-psychotic relatives and is relatively stable. A recent unitary model, following Euger Bleuler, proposes that schizophrenia may be a single disorder linked by a common pathophysiology, which leads to a misconnection syndrome of neural circuit. This disruption in the fluid coordination of mental activity that is the hallmark of normal cognition, can produce diversity of symptoms. The individual with mistimated information transfer may incorrectly connect perceptions and associations and misinterpret both external and internal processes, leading in turn to delusions or hallucinations. Defects in coordinating language production will lead to "though disorder." Difficulties in inhibiting or prioritizing may also lead to the various negative symptoms. We performed a study to characterize patients with schizophrenia (DSMIV-TR) on the basis of their clinical features and their deficits in specific domains of cognition.

*Methods*. Ninety-six outpatients were included in the study. Assessment instruments were: a semistructured interview; specific rating scales for psychopathology of schizophrenia, for depression and for social functioning; and a neupsychological battery including the WCST and the CPT.

*Results*. Impaired executive functions have been found to be associated with positive symptoms, impaired sustained attention with negative symptoms while depressive symptoms showed no correlation with specific cognitive domains.

*Conclusion*. There is abundant evidence that neuropsychological dysfunction is a core deficit and a phenotypic marker in schizophrenia.

## POSTERS

### 347P. Melanotic Neuroepithelial Tumor of Infancy

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This tumor, also known as melanotic prognonoma, was described in 1966 by Borello and Gorlin in the maxilla and they thought that it originated from neuroblastic elements. Infants are mainly affected, are locally invasive but in general they behave as benign tumors.

*Presentation of the case*. In 2002 a one-year-old girl developed tumor in the right parietal region, initially diagnosed in another hospital as alveolar rhabdomyosarcoma. It was subtotally resected and the child was referred to the Mexico City General Hospital for chemotherapy, which was discontinued because of neurologic deterioration culminating in death. The autopsy revealed a mass that infiltrated soft tissues, bones and dura mater. The temporal lobe, diencephalic structures and posterior limb of the internal capsule were compressed. The ventricular system was dilated.

The tumor was solid, dark blue, with whitish gray areas. It was composed of small cells in an alveolar pattern, and gland like structures in a fibrillary network. Many cells contained melanin. Positivity for synaptophysin and neurofilament protein was demonstrated. Cytokeratin and epithelial membrane antigen were positive in the pseudoglandular areas.

*Conclusion*. This tumor is infrequent, occurs mainly in the maxilla, but has been reported in the anterior fontanelle, dura mater, cranial cavity, long bones and epididymis. Its origin is controversial, most evidence points to the neural crest.

### 348P. Intraspinal Endodermal Cyst: Ultrastructural Study of Abnormal Cilia

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*Background*. Intraspinal endodermal cysts are rare benign lesion. Six cell types of the lining epithelium have been identified: ciliated cells, non-ciliated cells, goblet cells, basal cells, kulchitsky cells, and undifferentiated cells. The cell types and their topographic distribution are similar to that of the bronchial epithelium.

*Objective*. The purpose of this report is to describe the detailed morphological alteration of the cilia of intraspinal endodermal cyst and compare the findings with the ciliary abnormalities of the brochial epithelium of various diseases.

*Methods*. Specimens of the surgically removed intraspinal endodermal cyst at the cervico-thoracic region from 3 patients (21/F, 26/M, 36/F) were submitted for ultrastructural study.

*Results*. A wide spectrum of ciliary abnormalities were found: *i)* cilia with abnormal axonemal distribution, *ii)* swollen cilia, *iii)* compound cilia with or without excessive ciliary matrix, *iv)* naked cilia without limiting membrane, and *v)* intracytoplasmic cilia and aggregates of microtubules. Of these, compound cilia and swollen cilia were most common. Cilia with dynein arm deficiency were not observed. Ciliary abnormalities found in this study were very similar to those described in the bronchial epithelium of various diseases.

*Conclusion*. *i)* Ciliary abnormalities of intraspinal endodermal cyst are common. *ii)* The epithelium of intraspinal endodermal cyst shares cilio-genesis and susceptibility to abnormal ciliary formation similar to that of the bronchial epithelium. *iii)* In the absence of exogenous irritations, the abnormal ciliary formation may be related to the pressure effect and noxious irritation of the luminal secretions.

### 349P. Colloid Cyst in the Cerebellum

Sipõcz G, MD; Kovács Z, MD

The authors report the case of a 46-year-old female patient with tumor in the cerebellum near to the fourth ventricular cerebri. They review the clinical symptoms, the diagnostics, the method of surgery and present the MR and CT findings and the histology of the tumor. The detailed histology certified the colloid cyst. They emphasize that fact this type of the tumor was known only in the third ventricular cerebri in the literature till now.

*Conclusion*. The colloid cyst may appear not only in the third ventricular cerebri. This will the first report of so kind of colloid cyst in the literature.

### 350P. Primary Frontal-Lobe Germinoma Appearing as a Large Cyst. Case Report

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*Background*. Primary cerebral lobe germinoma may be rare. A number of cases reported previously have not been examined by modern brain imaging techniques, and hence the exact localizations or extensions of the tumors still remain unclear. We describe here an autopsy case of an intracranial germinoma that arose primarily in the right frontal lobe of a 20-year-old man.

*Case report*. On January 26, 2001, he suddenly developed respiratory arrest, and was transferred to our hospital. CT scans revealed a well demarcated, low-density lesion in the right frontal white matter. No enhancement in or around the lesion was present in postcontrast CT scans. He died 4 days after.

*Autopsy findings*. The brain, which weighed 1270 g before fixation, was autolytic and edematous with bilateral uncal and tonsillar herniations. A large cyst was found in the right frontal lobe. Histological examination disclosed the tumor to be germinoma with the typical 2-cell pattern. The tumor cells were immunoreactive for placental alkaline phosphatase.

*Conclusions*. A review of the literature yielded no previous autopsy reports of primary cerebral germinoma. The present case draws further attention to the peculiar radiographical features of this tumor, which is radiosensitive and potentially curable.

### 351P. Spheno Occipital Chordoma. Report of Three Autopsy Cases

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Chordoma are interesting from histological, biological and embryological view points. Chordomas are rare, constituting 0.2% of intracranial tumors.

*Case 1*. A 48-year-old female. *Case 2*. A 26-year-old male. In both cases a lobulated mass was found over the ventrolateral surface of the medulla. Compression of the cranial nerves VIII, IX, X, XI and XII was noted. In case 1 there was, in addition, involvement of the suboccipital nerve of Arnold, which explained the sharp pain in the area. In these 2 cases the hospital stay was brief due to the severity of the cilinical picture. Death was due to respiratory failure.

*Case 3* was that of a 52-year-old female with a large tumor on the ventral surface of the midbrain, pons and medulla. It was resected almost in its entirety using a transclival approach. She died in the immediate postoperative period. Death was attributed to surgical damage of the medulla.

In the 3 cases the clinical manifestations allowed the neurologists to make the diagnosis of neoplasm adyacent to the foramen magnum with compression of the medulla and last cranial nerves.

All are typical examples of chordomas originated in the rostral end of the notochord.

### 352P. Brain Metastases with Unknown Primary Tumor

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*Objectives*. Brain metastases are common as the first symptom in systemic cancers. we intended to explore the clinical and histopathological aspects, and frequency of brain metastases with unknown origin.

*Methods*. One hundred and five consecutive patients (mean age 52.6) with brain metastasis admitted to 4 neurosurgical centers of TUMS (1992-2002) were studied. Age, sex, site of the metastasis, neurological manifestations, histopathological pattern, origin, survival and management were investigated. Fifty-three (50.5%) of the cases had previously undiagnosed primary tumors.

*Results*. Forty-seven (88.7%) of the 53 cases (male/female: 2.1:1), had solitary lesions. Local paresis was the most frequent symptom. On pathological exam, the primary tumors were: lung (30.2%) (adenocarcinoma and squamous carcinoma) and breast (1.9 %) compared with 5.8% and 30.8% respectively in those with known primary tumors. Kidney ,colon and the less common were: choriocarcinoma, Ewing carcinoma, and germ cell tumor. The primary origin remained unknown in 17 (32.1%), of which 7 were adenocarcinoma, 5 Squamous carcinoma and 5 undifferentiated metastatic cells. The mean survival was 11 months in all 105 cases compared to 17 months in 53 cases with unknown primary.

*Conclusion*. The pathology, frequencies and survival of brain metastasis are different between the 2 groups with unknown and known primary tumors. This may be because of the short brain metastasis interval of lung tumor compared with tumors such as breast.

### 353P. Brain Metastasis of Cardiac Myxoma. An Example of the Diagnostic Relevance of the Patient's Clinical Information

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True metastasis of morphologically benign cardiac myxoma in the brain is a rare event. Because some cases may present before the primary tumor has been clinically identified, or a past history of cardiac surgery may not have been disclosed to the neuropathologist at the time of diagnosis, these tumors may be mistaken for vascular neoplasm.

We report the case of a 72-year-old man, who presented with a one-week history of headache, nausea and vomiting. Past history included a non-specified cardiac surgery, performed at an outside hospital two years before. MRI revealed an isolated, 3 cm of diameter, enhancing mass lesion in the left cerebellar hemisphere. Microscopically, a well-defined, lobulated, myxoid lesion containing small nests and short strands of elongated and epithelioid bland cells, showing prominent vasoformative features, was observed. Tumor lobules were separated by thick fibrotic tracts with prominent hemorrhage signs, both old and recent, and focal calcification. Mitotic figures were not seen, and there was no necrosis. The MIB-1 labeling index was <1%. The perivascular cells were strongly reactive for CD31, SMA, alpha-1-antitrypsin, and vimentin, and were negative for citokeratins, EMA, GFAP, synaptophysin, and S-100 protein, simulating an epithelioid hemangioendothelioma. The correct diagnosis was achieved only after an active investigation of the patient's cardiac history.

The possibility of a metastatic cardiac myxoma should be considered in the differential diagnosis of any vascular and myxoid, low-grade tumor in the brain of any adult patient. Although brain metastasis can appear in the setting of a well-known cardiac history, more frequently, it can be the initial manifestation of an occult cardiac myxoma. The present case illustrates the difficulty in recognizing some extremely uncommon entities and stresses the importance of the clinical history in surgical neuro-oncology.

## POSTERS

### 354P. Comparison Between the Analgesic Effect of Carum Copticum Extract and Morphine in Phasic Pain Model in Mice

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*Introduction*. Pain is a universal complaint which needs further investigations for new pain relieving agents. *Carum Copticum* (Yamani Ajowan) is a plant in Umberella family which is mentioned to have some therapeutic effect on headache and joint pains in Iranian traditional literatures, but there is a little if any scientific reports to prove its effects on pain.

*Method*. We conducted to design an experimental clinical trail study to assess and compare the analgesic effect of ethanolic extract of *Carum Copticum* fruit with morphine by using a tail-flick analgesiometer device.

*Results*. Our results indicate that the test drug produced significant increase in tail-flick latency during 2 hours post drug administration ($p<0.05$). The peak of the effect was observed at 45 minutes after drug injection, which was comparable to that of one mg/kg morphine (ip). Positive results in this type of analgesiometric test indicate that the antinociceptive action may be of the opoid type.

*Conclusions*. The present study support the claims of Iranian traditional medicine showing that *Carum Copticum* extract possesses a clear-cut analgesic effect. However, further investigations are required to evaluate the efficacy and safety of this herbal medication in man.

### 355P. Behavioral Effects of Pyridostigmine (PB) Combined to Chronic Stress in Rats

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*Introduction*. It has been suggested that operational stress combined to PB, given to soldiers as prophylactic protection against potential soman release, might have had unexpected adverse health effects causing the symptoms of Gulf War Illness. We have developed an experimental model to determine the effects of this combination on cognitive and social behavior.

*Methods*. 160 male Wistar rats were divided in 2 groups: stressed and sham-stressed. The pole-climbing test was used from day 1 to day 5 and from day 8 to day 12 to induce chronic stress. One half of each group was given PB daily (1.5 mg/kg/day, po) 30 minutes before stress. One half of each subgroup was tested for learning and memory using water-maze applied from day 15 to day 19 and at days 52, 113 and 199. The second half was tested for social behavior using "electric fight" at the same delays. At the end of experiments brain areas were dissected out for acetylcholinesterase determination.

*Results*. PB combined to chronic stress induces learning and memory dysfunction; chronic stress alone induces aggressiveness and impulsiveness behavior which is increased by PB. These data suggest that PB combined to chronic stress might have delayed neurological effects.

### 356P. Influence of NIR-Therapy on Patients with Panic Attacks

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To correct autonomic disorders, we have exposed the body to narrow-range far-infrared radiation (NIR therapy). Infrared radiation has long been used in medical practice. Scientists at the Materials Science Institute affiliated with the Uzbek Academy of Sciences have built energy conversion systems based on ceramics that are capable of absorbing broad-spectrum electromagnetic radiation and emitting in a narrow far-infrared range. We observed 60 patients with panic attacks (PA). During a session of exposure to infrared emitters they notice general relaxation, sleepiness, and some even fall asleep and have dreams. They comment on a clearly pacifying effect of the treatment. A different picture emerges in patients with PA of hereditary etiology. This type is harder to cure—the complaints can go away only to reappear later. However, their intensity decreases all the same. Patients with PA stemming from nervous system disorders (more often it is vertebral osteochondrosis) and those of internal organs have good treatment outcomes. Neuroendocrine, motivative, psychovegetative, algesic psychopathologic symptomatology as decreased significantly in all patients with positive effect together with the improvement of the objective psycho physiologic indices. Intrahemispheric interactions were also improved, exactly: the power of frequency EEG spectrum increased, in general, because of both the increase of the slow rhythms from both sides and the approach of the coefficient of asymmetry to the control. Thus, the research conducted has demonstrated that on the whole resonance infrared therapy has a relaxing effect on the psycho autonomic system in both the norm and pathology. The data obtained show that infrared irradiation can be considered an effective method of non-drug therapy for PA.

### 357P. The Effect of Chamomill Extract on Phasic Pain in Mice

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Pain is an almost universal experience. Not surprisingly, its mechanisms of induction, transduction and sensation and the way to relief or attenuate pain sensation have been the object of considerable attention not only by neurophysiologist but also by philosophers, theologians and physicians since old time. Due to wide spread use of antinociceptive, therapeutics and suggested their side effects, recently more attentions have been focused on traditional alternatives.

Chamomill sp is referred to as a pain relief herbal therapeutic in Iranian traditional medicine literatures.

We conducted to study the effect of Chamomill extract on phasic pain induced by noxious thermal stimulus by using a tail-flick analgesiometer device in Syrian male mice weighting 32 to 38 g. Percent of analgesia index (%AI) was measured as indicated by D-Amour in 1941 in both test and control groups.

Our results indicate that Chamomill extract significantly attenuated pain sensation during a range of 60 to 90 minutes after intrapretoneal injection ($p< 0.05$). This analgesic effect was also com-

pared with doses of 0.5, 1.0 and 2.0 mg/kg injected subcutaneously which was comparable to one mg/kg morphine after 60 minutes following injections. We concluded that Chamomill extract induce a relatively powerful analgesic effect on phasic pain.

### 358P. The Analgesic Effect of Ethanolic Extract of Lactuca Sativa Seeds in Mice

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*Introduction*. Seed of *Lactuca Sativa* is an ancient drug that is mentioned by Iranian old physicians as an important drug for treating acute inflammation, chest pain, fever, headache, cough, etc. It is widely used in old Greek medicine for nervous disorders. But seeds of *Lactuca Sativa* were not scientifically studied for neuropharmacological activities in systemic manners.

*Methods*. It was planned to study the drug pharmacologically to substantiate an improve its therapeutic uses on scientific basis. In the present study a comprehensive pharmacological screening of the 50% ethanolic extract of *Lactuca Sativa* seeds was carried out for its effects on phasic pain. The drug was administered interapretoneally in a dose corresponding to human dose described in Iranian classical literature. Analgesiometer tail-flick test was carried out for detecting analgesia.

*Results*. In this analgesiometer test, the test drug did not produced significant increase in the reaction time (tail-flick latency) ($p = 0.453$).

*Conclusion*. The present study did not support the claims of Iranian traditional medicine in this type of analgesiometric test. However, it needs further investigations to assess the analgesic effect of this traditional herbal medication in other types of pain models.

### 359P. Functional and Metabolic States in Nervous Tissue

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The purpose of research was experimental differentiating and studying of the basic functional states of a nervous tissue at development of brain compression injury, focal brain ischemia and action of neurotropic drugs.

On the character of the change of the DC potential, EEG and local CBF, we define 3 basic functional and metabolic states of the nervous tissue: *i)* The state of the reduced metabolism accompanying with the DC positive shifts, the decrease in the EEG and the reduction in the local CBF. The similar state is met at the action of the majority known neuroprotective, somnolent and sedative preparations, and also at the satisfaction of food motivation, during NREM sleep. *ii)* The state with the usual level of metabolism, CBF and EEG amplitude. *iii)* The state of the increased metabolism accompanying with the DC negative shifts, the initial increase (at transition from a usual state) with the subsequent decrease in the amplitude of the EEG and increase in the local CBF. This state is typical for the majority of cases of the brain injury, the hypoxia, the ischemia, and also for the action most inhalation anaesthetics and neurotoxins. This concept allows to explain mechanisms and to predict changes functional and a metabolic state of nervous tissue at action of chemical and physical factors, and also development the hypertrophy and the hyperplasia, adaptations to injuring factors and mechanisms development of "tolerance" to the subsequent after transitive depolarization to injury.

### 361P. Anti Nociceptive Effect of Carum Copticum in Mice

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The C*arum Copticum* is a species of Umbeliferae family that its effective agents are an essence which is obtained from its fruits. This essence is colorless or mildly brownish and smells as thymole. The fruit of this plant have been traditionally used in many societies as an anti- inflammatory and pain relevant agent but there is no scientific report about these effects. So we designed an experimental study to investigate the effect of alcoholic extraction of *Carum Copticum* fruit on tonic pain induced by formalin in mice. Our result showed that 10 mg/kg of this extract decreased the pain in second phase, but not in first phase of formalin test. This effect in second phase was not significantly different from that of one mg/kg morphine. The comparison between the Anti nociceptive effect of Chamomile extract and morphine in mice. Chamomile is a plant in which many traputic effects has been mentioned since all time. One of its uses in Iranian traditional medicine was to relieve spastic pain. Since there is a little scientific documentation to prove its effectiveness in relieving pain, we conducted to study the effect of its extract on tonic pain induced by formalin in mice our result showed that 2 mg/kg Chamomile extract (I.P) attenuated the pain score in both phase of formalin test which was more prominent in second phase and was comparable that of morphine one mg/kg (I.P). In conclusion, Chamomile extract may act as an antinociceptive in a manner which resemble morphine analgesia.